Characterising sensory interactions between volatile phenols and other taint-causing compounds in South African red wines

by

Marianne McKay

Dissertation presented for the degree of
Doctor of Philosophy (Agricultural Sciences)

at

Stellenbosch University
Department of Viticulture and Oenology, Faculty of AgriSciences

Supervisor: Dr Astrid Buica
Co-supervisor: Prof Florian Bauer

April 2019

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated) that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 6 December 2018

Summary

South African red wine competes for limited market space, and must maintain consistent, excellent quality. One of the important modalities in assessing the quality and typicality of wine is odour perception (Hopfer et al., 2015). Most investigations quantify aroma compounds by chemical/analytical means and compare levels to odour detection thresholds (ODTs) provided by scientific literature. If malodourous compounds are present at concentrations above their ODTs, they may be considered a threat to wine quality as they exhibit odour activity values >1 (Louw et al., 2010; Prida & Chatonnet, 2010). A review of the literature reveals that studies in wine can use inappropriate ODTs for work carried out in a new wine matrix, and matrix effects on the activity and perception of a compound are often ignored. To properly scientifically evaluate the effect of any compound to wine aroma, formal sensory evaluation in the study matrix is essential (Villamor & Ross, 2013; Perry & Hayes, 2016).

Certain volatile phenols (VPs) can contribute to a continuum of smoke-taint related off-flavours including 'burnt', 'bretty', 'smoky', and 'ashy' attributes in wine (Jiranek, 2011; Kennison et al., 2011) at higher levels but are generally accepted as being benign to wine aroma at sub-threshold levels (Boidron et al., 1988; Prida & Chatonnet, 2010). Useful sensory studies on VPs in wine have been carried out (Simpson et al., 1986; Boidron et al., 1988; Chatonnet et al., 1992; Kennison et al., 2008; Petrozziello et al., 2014), but at supra-threshold levels, and the effect of combinations of subthreshold levels of VPs is not taken into account.

The main aims of this research were to characterise the sensory contribution of specific VPs at low levels to red wine odour, and to assess their effect on the perception of attributes through interactions with themselves and other compounds known to be involved in specific wine off-flavours. Chapter 2 addresses key knowledge and gaps within the literature around origin, perception and odour detection threshold of VPs associated with specific off-flavours (including 'smoke' and 'ashiness'), and previous studies concerning interaction of aroma compounds which may have relevance to the current study. Chapter 3 investigated trends within the chemical (GC-MS) and sensory (Descriptive Analysis) results for twelve commercial samples of smoke-affected wine. Associations between negative attributes and bushfire events prior to harvest were found. Results also showed that certain sensory effects could have resulted from combinations of subthreshold levels of VPs. The need arose to formally test perceptual interactions at subthreshold levels to see if various effects could be explained.

It was thus decided to investigate effects of three VPs: guaiacol, ortho-cresol, 4-ethylphenol, and two compounds associated with certain off-flavours in wine, viz. 3- isobutyl-2-methoxypyrazine (IBMP) and 2,4,6-trichloroanisole (TCA). The work was carried out in partially

de-aromatised Shiraz, and it was necessary to establish if ODTs provided in the literature were appropriate for this matrix. Formal sensory work is known to be complex, time-consuming and expensive, particularly in establishing ODTs, and thus a pragmatic sensory approach to the work is outlined in Chapter 4.

Chapters 5 and 6 address the perceptual effects of combinations of two, three, four and five off-flavour compounds on red wine aroma, which has not been conducted on this scale before. The results of this DA sensory study showed olfactory opposition between clean controls, wines spiked with single compounds (generally fruity and sweet-associated), and wines spiked with complex combinations of VPs and IBMP (linked to negative attributes). Chapter 7 demonstrates another pragmatic approach, using projective mapping (PM) with a large sample size (n=18). Comparable results to the DA interaction study for four compounds in red wine were shown. Chapter 8 investigates effects of combinations of two VPs on four cultivars in order to establish whether there were perceptual olfactory effects that were common to all cultivars, or whether the matrices responded differently from an aroma perception perspective, and shows that samples spiked with combinations of VPs and IBMP show consistently negative olfactory attributes that are independent of cultivar.

This research contributes to the sensorial and chemical characterization of selected VPs in red wines, and shows that subthreshold levels of VPs in combination with very low levels of IBMP and TCA can lead to olfactory interactions that cause various olfactory effects, some of them negative. This may help inform winemaking decisions, particularly when dealing with smoke-affected grapes, and/or cultivars that naturally have higher levels of methoxypyrazines, like Merlot and Cabernet Sauvignon. This study also emphasises the importance of understanding effects of VPs on wine aroma, and escalating awareness and sensitivity to these issues in the wine industry.

Opsomming

Suid-Afrikaanse rooiwyn kompeteer vir beperkte markruimte en moet uitstekende gehalte op ’n konsekwente basis onderhou. Een van die belangrikste modaliteite wanneer die gehalte en tipiese aard van wyn geassesseer word, is reukwaarneming (Hopfer et al., 2015). Die meeste ondersoeke kwantifiseer reukverbindings chemies/ analities en vergelyk vlakke met reukwaarnemingsdrempels (odour detection thresholds (ODT’s)) wat in die wetenskaplike literatuur verskaf word. Indien onwelriekende verbindings in konsentrasies hoër as hulle ODT’s teenwoordig is, kan hulle as ’n bedreiging vir wyngehalte beskou word as hulle reuk-aktiwiteitswaardes (odour activity values) van > 1 vertoon (Louw et al., 2010; Prida & Chatonnet, 2010). ’n Literatuuroorsig toon dat studies van wyn ongepaste ODT’s kan gebruik vir werk wat in ’n nuwe wynmatriks uitgevoer word, en in baie gevalle word matriks-effekte op die aktiwiteit en waarneming van ’n verbinding geïgnoreer. Om die effek van enige verbinding op wynaroma behoorlik wetenskaplik te evalueer, is formele sensoriese evaluering in die studiematriks noodsaaklik (Villamor & Ross, 2013; Perry & Hayes, 2016).

Sekere vlugtige fenole (VF’e) kan bydra tot ’n kontinuum van rooksmaak-verwante wangeure, insluitend ‘gebrande’, ‘brett-agtige’, ‘rokerige’ en ‘asagtige’ kenmerke, in wyn (Jiranek, 2011; Kennison et al., 2011) teen hoër vlakke, maar wat oor die algemeen aanvaar word as onskadelik vir wynaroma teen vlakke onder die drempel (Boidron et al., 1988; Prida & Chatonnet, 2010). Nuttige sensoriese studies oor VF’e in wyn is onderneem (Simpson et al., 1986; Boidron et al., 1988; Chatonnet et al., 1992; Kennison et al., 2008; Petrozziello et al., 2014), maar teen vlakke effe hoër as die drempel, en die effek van kombinasies van hierdie VF’e onder die drempel is nie in ag geneem nie.

Die vernaamste doelwitte van hierdie navorsing was om die sensoriese bydrae van spesifieke VF’e teen lae vlakke tot die reuk van rooiwyn te karakteriseer, en om hulle effek op die persepsie van eienskappe afkomstig van interaksies met mekaar en met ander verbindings wat in spesifieke wyn-wangeure betrokke is, te assesseer. Hoofstuk 2 ondersoek belangrike kennis en gapings in die literatuur oor oorsprong, waarneming en die reukwaarnemingsdrempels van VF’e wat gekoppel word aan spesifieke wangeure (insluitend ‘rook’ en asserigheid’), en vorige studies oor die interaksie van aromaverbindings wat moontlik relevant is vir die huidige studie. Hoofstuk 3 ondersoek tendense in die chemiese (GC-MS) en sensoriese (beskrywende analise, BA) resultate vir twaalf rookgeaffekteerde kommersiële wynmonsters. Verbintenisse is gevind tussen negatiewe kenmerke en bosbrande voor oes. Die resultate toon ook dat sekere sensoriese effekte kon ontstaan het uit kombinasies van sub-drempel vlakke van VF’e. Die behoefte het ontstaan om perseptuele interaksies teen sub-drempel vlakke formeel te toets om te sien of die verskillende effekte verklaar kon word.

Daar is dus besluit om die effekte van drie VF'e te ondersoek: guajakol, orto-kresol, 4-etielfenol, en twee verbindings gekoppel aan sekere wangeure in wyn, nl. 3-isobutiel-2-metoksipirasien (IBMP) en 2,4,6-trichloro-anisool (TCA). Die werk is uitgevoer op Shiraz waarvan die aroma gedeeltelik verwyder is, en daar moes eers bepaal word of die ODT's wat in die literatuur verskaf word, toepaslik was vir hierdie matriks. Formele sensoriese werk is kompleks, tydrowend en duur, veral wanneer ODT's bepaal word, en 'n pragmatiese sensoriese benadering tot die werk word dus in Hoofstuk 4 uitgestippel.

Hoofstukke 5 en 6 kyk na die perseptuele effekte van kombinasies van twee, drie, vier en vyf wangeurige verbindings op rooiwyn-aroma, wat nie tevore op so 'n groot skaal uitgevoer is nie. Die resultate van hierdie BA- sensoriese studie toon reukverwante opposisie tussen skoon kontroles, wyn waarby een verbinding gevoeg is (gewoonlik vrugtig en soet assosiasies), en wyn waarby komplekse kombinasies van VF'e en IBMP (wat verband hou met negatiewe kenmerke) gevoeg is. Hoofstuk 7 demonstreer nóg 'n pragmatiese benadering, waarvoor projective mapping (PM) met 'n groot monster (n = 18) gebruik is. Resultate wat vergelykbaar is met die BA-interaksie studie is vir vier verbindings in rooiwyn getoon. Hoofstuk 8 ondersoek die effekte van kombinasies van twee VF'e op vier kultivars om te bepaal of daar perseptuele reuk-effekte was wat algemeen is aan alle kultivars en of die matrikse verskillend gereageer het vanuit 'n aromawaarnemingsperspektief. Daar is gevind dat monsters waarby kombinasies van VF'e en IBMP gevoeg is, konsekwent negatiewe reukkenmerke getoon het wat onafhanklik was van kultivar.

Hierdie navorsing dra by tot die sensoriese en chemiese karakterisering van die gekose VF'e in rooiwyn en wys dat VF-vlakke onder die drempel, in kombinasie met baie lae vlakke van IBMP en TCA, kan lei tot reuk-interaksies wat 'n verskeidenheid reuk-effekte kan veroorsaak, waaronder negatiewe effekte. Dit kan help om wynbereidingsbesluite in te lig, veral wanneer daar gewerk word met druiwe wat deur rook geaffekteer is en/of met kultivars wat natuurlik hoër vlakke van metoksipirasiene het, soos Merlot en Cabernet Sauvignon. Hierdie studie beklemtoon ook die belangrikheid van 'n begrip van die effekte van VF'e op wynaroma, en van 'n verhoging van bewussyn van en sensitiwiteit vir hierdie kwessies in die wynbedryf

This dissertation is dedicated to my father, **Christopher Brian Seward Case** who taught me to question everything. It is also dedicated to my friend **Michaela Eva Bojé** who taught me the most important answers.

Biographical sketch

Marianne Alison McKay is a lecturer in Oenology at Stellenbosch University, specialising in teaching and learning (T&L), and the science of wine aroma. She has a BSc in Chemistry and Geography from UCT, and was the first woman to graduate with MSc (Agric) in South Africa. She worked in Analytical Chemistry at the University of Cape Town for six years as a Senior Scientific Officer, running the ICP and HPLC services until 1996, and then in London, United Kingdom where she worked as an Operations Manager in clinical trials and validation services for pharmaceutical companies. She returned to academia as a lecturer in Wine Sciences at Plumpton College, Brighton, UK in 2000. Since her return to South Africa in 2007, she has been at Stellenbosch University (SU). Her research has taken her from volatile phenols, smoke and related taints to engaged learning methodologies and sensory evaluation. Her T&L research now has a strong focus in work-integrated learning and transformation in science. She won the national South African Council for Higher Education Excellence in Teaching Award in 2015, and was awarded a Teaching Fellowship at Stellenbosch University (2016 - 2019) and is an SU Distinguished Teacher (2017). She has a son, David, and lives in Somerset West.

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- My supervisor, Dr Astrid Buica, for her excellent support, brisk feedback and scientific guidance. Without her, this task would not have been achieved.
- My co-supervisor, Professor Florian Bauer, for his support and ability to cut to the scientific heart of each issue.
- Mrs Valeria Panzeri who drove the practical sensory aspects with expertise and for being such a skilful and efficient panel leader
- Professor Martin Kidd for his expertise with statistical analyses and patience with my constant questions and emails.
- Mrs Jeanne Brand for assisting with sensory ideas and last minute statistical diagrams.
- Mr Lucky Mokwena, for his prompt assistance with chemical analyses.
- Ms Karin Vergeer for her administrative assistance and cheerful encouragement.
- All the sensory panelists for their reliability and sensitivity to volatile phenols.
- And very importantly, my wonderful son, David Case, for putting up with his mother for the last two years. It has not been easy, I know.

Preface

This dissertation is presented as a compilation of 9 chapters. Each chapter is introduced separately and is written according to the style of the journal to which iwas submitted for publication.

Chapter 1	General Introduction
Chapter 2	Literature review
Chapter 3	Elucidating Chemical and Sensory Effects of Volatile Phenols in Smoke-Affected Red Wines
Chapter 4	Testing the Sensitivity of Potential Panelists for Wine Taint Compounds Using a Simplified Sensory Strategy
Chapter 5	Perceptual interactions and characterisation of odour quality of binary mixtures of volatile phenols and IBMP in a red wine matrix
Chapter 6	Investigation of olfactory interactions of low levels of five off-flavour causing compounds in a red wine matrix
Chapter 7	Research results 1 Comparison of Descriptive Analysis vs Projective Mapping for characterising interactions between four taint compounds
Chapter 8	Research results 2 Investigating the effects of two volatile phenols on aromatic perception of four red wine cultivars using Projective Mapping
Chapter 9	General discussion and conclusions

Table of Contents

Chapter 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Data for 2017 show that South Africa occupied the eighth position in the global market in terms of wine production volume, but only the eleventh position in terms of financial value of wine sales internationally (OIV, 2018). Thus, room for expansion and increase in revenue exist, as long as the quality of the South African offering matches or out-performs that of its international competitors. Wine aroma is arguably the most important intrinsic factor used by consumers to judge wine quality (Wilson *et al.*, 2018), and off-flavours in SA wine would thus threaten viability of sales in the uncompromising global market. Research that provides insight into the sources, effects and amelioration of off-flavour in wine is therefore crucial in supporting the SA wine industry in improving the quality of its products.

The perception of wine aroma is the result of a number of different factors including the composition of volatile compounds in the wine (Barbe *et al.*, 2008; Sáenz-Navajas *et al.*, 2010; Ferreira, 2012), the perceptual interactions between the volatiles (Yang, 2017; Wilson *et al.*, 2018), the physical and chemical effects of non-volatile components of the wine matrix (Styger *et al.*, 2011; Villamor & Ross, 2013; Sáenz-Navajas *et al.*, 2015), and the ability and experience of the person perceiving the aroma (Brown *et al.*, 1968; Chrea *et al.*, 2004; Ferdenzi *et al.*, 2014; Thomas-Danguin *et al.*, 2014; Silva Teixeira *et al.*, 2015).

Volatile phenols (VPs) are a class of compounds that can be found in wine at low levels, but may have a potent olfactory effect because of low odour thresholds (Parker *et al.*, 2012; Krstic *et al.*, 2015). VPs in wine have been shown to come from several sources including microbial, toasting of oakwood, as well as grapes and smoke taint. Characterisation of some of the sensory effects of VPs has been carried out (Botha, 2010; Parker, *et al.,* 2012; Panzeri, 2013; Tempere *et al.*, 2016), and in a limited number of cases, the odour detection thresholds (ODTs) have been confirmed (Czerny *et al.*, 2008; Parker *et al.*, 2012). It has been found that sensory results do not always correlate well with predictions that are based on the chemistry of the solution (Villamor *et al.*, 2013; Lapalus, 2016; Wilson *et al.*, 2018).

With recent heatwaves, and more frequent and extensive forest fires across the world (Fried &Torn, 2004; Strydom & Savage, 2016; Wolf, 2018), it seems likely that increasing importance will be placed on understanding the effects of smoke on agricultural crops. A study of the composition of commercial wines affected by naturally-occurring (i.e. not experimentally induced) smoke events would be very useful in establishing the impact of bushfires on the chemical (VP concentrations) and sensory profile of red wine, as this research is rare in the literature.

Detection thresholds found in model solutions are not comparable to those determined in wine, and odour thresholds in any matrix other than the study matrix may be irrelevant, as suggested by Meilgaard *et al.*, (2016). Many VPs have not yet been characterised in any wine matrix or assigned threshold values in any formal sensory study. A significant gap thus exists in the literature as regards matrix effects on VPs, specifically the effect of the VP composition on different cultivar aroma profiles. Knowledge of the chemical and sensory effects in wines containing VPs in combination with other compounds would give greater understanding of the nature of off-flavours. Although anecdotal data suggests that these compounds contribute to 'acrid' and 'green-associated' attributes, there are no studies, to our knowledge, focused on olfactory perception of interactions between VPs and other taint-causing compounds like IBMP and TCA.

Sensory methodologies that can be used to suit specific experimental objectives and elucidate wine odour have been described and implemented, but the field continues to evolve and new methods are still being evaluated. Rapid sensory methodologies in particular have recently received attention in the primary and popular wine literature, but additional research is needed to fully describe the applicability of particular methods to various scenarios in wine tasting. Rapid methods like projective mapping and sorting are pragmatic approaches given the fairly complex and time-consuming standard practice of descriptive analysis (DA), but would still need to be tested against this 'gold standard' in sensory evaluation.

This study focuses on the perceptual olfactory interactions of five aroma compounds associated with taints in red wine, namely guaiacol, *ortho*-cresol (*o*-cresol), 4-ethylphenol (4-EP), 3-isobutyl-2-methoxypyrazine (IBMP) and 2,4,6-trichloroanisole (TCA). Such a holistic approach has not been attempted with as many compounds in a single sensory experiment. A de-aromatised red wine as recommended by Pineau, *et al*, (2009) was used for studies looking at potential masking and synergistic interactions, and different cultivars were tested to elucidate matrix effects on interactions, as well as the effects of compounds on the aroma profiles of different cultivars. The study aimed at contributing to our current understanding and providing novel insights regarding descriptors and odour thresholds of VPs in red wine. This new knowledge should prove useful to the South African and global wine industries affected by smoke taint and deepen our understanding of chemical and sensory effects of VPs. Ultimately, the aim would be to produce wines that satisfy the rigorous demands of the global market despite escalation of wildfires globally.

Research aims

The aims and objectives of this project are:

1. **To investigate the volatile phenol chemical and sensory profiles of commercial samples of potentially smoke-affected South African red wines.**
    a) To chemically characterise the volatile phenols levels by GC-MS in a number of commercial samples that have been earmarked as potentially smoke-tainted
    b) To sensorially characterise the same wines with Descriptive Analysis using a trained panel
    c) To elucidate relationships between the chemistry and the sensory in these wines, and establish what attributes can and cannot be explained by the VP chemistry

2. **To investigate the ODTs of compounds of interest in a red wine matrix**
    a) To confirm the sensitivity of potential sensory panel members to compounds at ODT level in a specific red wine matrix using a simplified version ASTM E-679-04 triangle test
    b) To establish if previous training in sensory evaluation of wine influenced panellists' abilities to distinguish threshold differences between samples and controls.

3. **To characterise the perceptual olfactory interactions caused by combinations of five odour-active compounds associated with specific off-flavour issues** (namely, guaiacol, *o*-cresol, 4-EP, IBMP and TCA) **in a red wine matrix**
    a) To characterise single and binary interactions of selected off-odour compounds in red wine
    b) To elucidate new sensory attributes resulting from perceptual blending in complex mixtures of five odorants in red wine
    c) Generate hypotheses for further volatile phenol/ taint compound studies by exploring trends in the results

4. **To explore the use of rapid sensory methods in interaction studies for volatile phenols** and validate the use of Projective Mapping for characterising combinations of off-odour compounds in a red wine matrix, by comparing results to those of a similar study using Descriptive Analysis.

5. **To address the importance of different red wine matrices in effecting olfactory perceptual interactions by** characterizing similarities and differences in attributes resulting from olfactory interactions due to the same combinations of volatile phenols in four different red wine matrices

References

Barbe, J., Pineau, B., *et al.*, 2008. Instrumental and sensory approaches for the characterization of compounds responsible for wine aroma; Chem. Biodivers. 5, 6, 1170–1183.

Botha, J., 2010. Sensory, chemical and consumer analysis of Brettanomyces spoilage in South African wines Master's Thesis, Faculty of Agrisciences, Stellenbosch University, Western Province, South Africa.

Brown, K., Maclean, C.M., *et al.,* 1968. The distribution of the sensitivity to chemical odors in man; Hum. Biol.40, 4, 456–472.

Chrea, C., Valentin, D., *et al.,* 2004. Culture and odor categorization: Agreement between cultures depends upon the odors; Food Qual. Prefer. 15, 7–8, 669–679.

Czerny, M., Christlbauer, M., *et al.,* 2008. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. Eur. Food Res. Technol. 228, 2, 265–273.

Ferdenzi, C., Poncelet, J., *et al*., 2014. Repeated exposure to odors induces affective habituation of perception and sniffing. Front. Behav. Neurosci. 8, 119.

Ferreira, V., 2012. Revisiting psychophysical work on the quantitative and qualitative odour properties of simple odour mixtures: a flavour chemistry view. Part 2: qualitative aspects. A review. Flavour Frag. J. 27, 3, 201–215.

Fried, J., Torn, M., *et al*., 2004. The Impact of Climate Change on Wildfire Severity: A Regional Forecast for Northern California Clim. Change 64, 1/2, 169–191.

Fudge, A.L., Ristic, R., *et al.,* 2011. Amelioration of smoke taint in wine by reverse osmosis and solid phase adsorption. Aust. J. Grape Wine Res. 17, 2, 41–48.

Hayasaka, Y., Baldock, G., *et al*., 2010. Glycosylation of smoke-derived volatile phenols in grapes as a consequence of grapevine exposure to bushfire smoke J. Agric. Food Chem. 58, 20, 10989–10998.

Kennison, K., Wilkinson, K., *et al.,* 2008. Smoke-derived Taint in Wine: Effect of Postharvest Smoke Exposure of Grapes on the Chemical Composition and Sensory Characteristics of Wine. J. Agric. Food Chem. 55, 26, 10897–10901.

Kennison K.R., Wilkinson, K.L., *et al.* (2011). Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties. Australian Journal of Grape and Wine Research 17(2), S5-S12.

Krstic, M., Johnson, D., *et al.,* 2015. Review of smoke taint in wine: Smoke-derived volatile phenols and their glycosidic metabolites in grapes and vines as biomarkers for smoke exposure and their role in the sensory perception of smoke taint Aust. J. Grape Wine Res. 21, 537–553.

Lapalus, E., 2016. Linking sensory attributes to selected aroma compounds in South African Cabernet Sauvignon wines, Master’s Thesis, Faculty of Agrisciences, Stellenbosch University, Western Province, South Africa.

Meilgaard, M.C., Civille, G., *et al*., 2016. Sensory evaluation techniques. (Fifth ed.). CRC Press, Taylor & Francis Group, New York.

Noestheden M, Romero-Montalvo *et al*., (2018) Detailed characterization of glycosylated sensory-active volatile phenols in smoke-exposed grapes and wine, Food Chem. 259 pp: 147-156

OIV, 2018. State of the Viniviticultural World Market (April 2018) France.

Panzeri, V., 2013. Influence of vineyard posts type on the chemical and sensorial composition of Sauvignon blanc and Merlot noir wines. Master’s thesis, Stellenbosch University, Western Province, South Africa.

Parker, M., Osidacz, P., *et al*., 2012. Contribution of Several Volatile Phenols and Their Glycoconjugates to Smoke-Related Sensory Properties of Red Wine. J. Agric. Food Chem 60, 2629–2637.

Sáenz-Navajas, M., Campo, E., *et al*., 2010. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. Food Chem. 121, 4, 1139–1149.

Sáenz-Navajas, M., Avizcuri, J., *et al*., 2015. Sensory-active compounds influencing wine experts' and consumers' perception of red wine intrinsic quality. LWT - Food Sci. Technol. 60, 1, 400–411.

Silva Teixeira, C., Cerqueira, N., *et al*., 2015. Unravelling the olfactory sense: From the Gene to Odor Perception. Chem. Senses 41, 2. DOI: 10.1093/chemse/bjv075

Strydom, S. & Savage, M., 2016. A spatio-temporal analysis of fires in South Africa. South African J. Sci. J Sci 112, 11, 1–8.

Styger, G., Prior, B., *et al*., 2011. Wine flavor and aroma. J. Ind. Microbiol. Biotechnol. 38, 9, 1145–1159.

Tempere, S., Schaaper, M.H., *et al*., 2016. The olfactory masking effect of ethylphenols: Characterization and elucidation of its origin Food Qual. Prefer. 50, 135–144.

Thomas-Danguin, T., Sinding, C., *et al*., 2014. The perception of odor objects in everyday life: a review on the processing of odor mixtures Front. Psychol. 5, June, 1–18.

Valentin, D., Chollet, S., *et al*., 2012. Quick and dirty but still pretty good: a review of new descriptive methods in food science Int. J. Food Sci. Technol. 47, 8, 1563–1578.

Villamor, R. & Ross, C., 2013. Wine Matrix Compounds Affect Perception of Wine Aromas Annu. Rev. Food Sci. Technol. 4, 1, 1–20.

Villamor, R., Evans, M., *et al*., 2013. Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine. Food Res. Int. 50, 1, 38–45.

De Vries, C., Buica, A., *et al*., 2016. The Impact of Smoke From Vegetation Fires on Sensory Characteristics of Cabernet Sauvignon Wines Made From Affected Grapes. South African J. Enol Vitic. 37, 1, 22-30

De Vries, C., Mokwena, L., *et al*., 2016. Determination of Volatile Phenol in Cabernet Sauvignon Wines, Made from Smoke-affected Grapes by using HS-SPME GC-MS. South African J. Enol. Vitic. 37, 1, 15–21.

Wilkinson, K.L., Ristic, R., *et al*., 2011. Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine Aust. J. Grape Wine Res. 17, S22–S28.

Wilson, C., Brand, J., *et al*., 2018. Interaction Effects of 3-Mercaptohexan-1-ol (3MH), Linalool and Ethyl Hexanoate on the Aromatic Profile of South African Dry Chenin Blanc Wine by Descriptive Analysis (DA) South African J. Enol. Vitic. 39, 2, 271–283.

Wolf, L., 2018. Wildfires and wine: A detective story Chem. Eng. News 96, 19, 22–25.

Yang, W., Li, W., *et al*., 2015. Odour prediction model using odour activity value from pharmaceutical industry Austrian Contrib. to Vet. Epidemiol. 8, 51–60.

Yang, Y., 2017. The influence of tannin and tannin with salivary protein on the volatility and the perceived intensity of ethyl hexanoate in a wine-like solution Lincoln University, Christchurch, New Zealand. Digital Dissertation for Fulfilment of Honours Degree in Viticulture & Oenology.

Chapter 2

LITERATURE REVIEW

LITERATURE REVIEW

Factors influencing olfactory perception of selected off-odour causing compounds in red wine: a critical review

This review of the current literature includes a brief discussion on the sources of selected off-flavour-causing compounds in red wine, and the attributes associated with them. Extensive work into the chemical aspects of off-flavour in wine has been carried out by international researchers, but not as many studies focus on the organoleptic effects. This literature review therefore has a focus on the status of sensory aspects, and tries to address what previous workers have found pertaining to the perception of these compounds in wine, as well as issues relating to judge/human perception and effects of the matrix. It will briefly cover the work that has been carried out in characterising perceptual interactions between compounds in red wine, and touch on some of the sensory approaches that are needed in order to investigate and characterise these issues.

1. Off-flavour-causing compounds in red wine

Due to the variability of odours in wine, volatiles are the components that often best define the parameters of quality and typicality (Mozzon *et al.*, 2016). Red wine can manifest a range of off-odours (unexpected and/or undesirable smells) as a result of the presence of volatile compounds that enter the wine production process from a number of different sources. These off-odours, which form a continuum of perceived aromas across a broad range of odour-families, frequently lead to a drop in perceived wine quality, and may contribute to lack of typicality of style or cultivar profile (Parr *et al.*, 2007). Relationships between quality, typicality and taint are not well documented in the literature. Descriptors associated with off-odour issues in red wine can include some easily identifiable problems like oxidation (Zoecklein *et al.*, 1995; Culleré *et al.*, 2007), reductive or sulphur-related problems (Shutz & Kunkee, 1977; He *et al.*, 2013; Franco-Luesma & Ferreira, 2016), microbial issues like 'brettiness' (Curtin *et al.*, 2008; Romano *et al.*, 2009; Botha, 2010; Tempere *et al.*, 2014) or cork taint (Van Eeden, 2009; Cravero *et al.*, 2015) and herbaceousness (Hein *et al.*, 2009; Suklje *et al.*, 2013; Lapalus, 2016).

The range and variability of taints and off-flavours in red wine involves hundreds of volatiles (Rauhut & Kiene, 2019), and it is impossible to address the impact of even a percentage of them in one review. This study will therefore concentrate on those that have been linked to 'smoky/ burnt' (Goode, 2008, Krstic, *et al.,* 2015), 'acrid' (Hammond, 2015), 'herbaceous'/'dusty' (Heyns, 2014), and 'burnt/rubber' (Bearak, 2009) off-flavours that have been associated with, but are not unique to, South African red wine. These can be seen in Figure 1, and are volatile phenols (specifically guaiacol, 4-ethyphenol, and *ortho*-cresol) and two other compounds: 3-isobutyl-2-methoxypyrazine and 2,4,6- trichloroanisole.

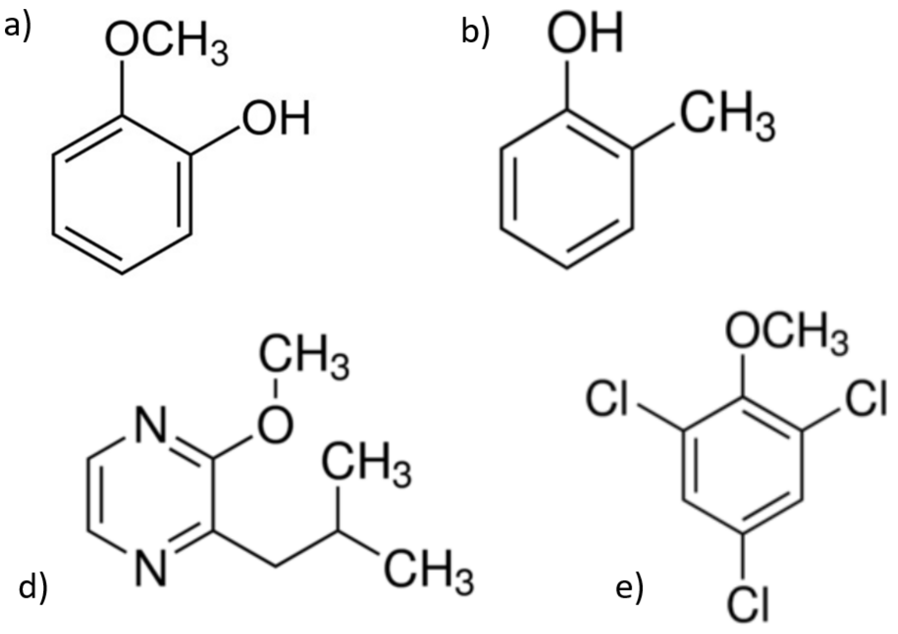


Figure 1. Selected taint-causing compounds in red wine: a) guaiacol b) ortho-cresol c) 4-ethylphenol d) 3-isobutyl-2-methoxypyrazine e) 2,4,6-trichloroanisole

1.1 Volatile phenols

Volatile phenols (VPs) are a group of compounds associated with a range of descriptors in wine (Boidron *et al.*, 1988; Parker *et al.*, 2012; Lorrain *et al.*, 2013), some of which are negative. The class of VPs includes sub-groups such as ethylphenols, vinylphenols, syringols, cresols and xylenols. The VPs most commonly found in red wine are 4-ethylguaiacol (4-EG), 4-ethylphenol (4-EP), 4-methylguaiacol (4-MG), vinylphenols, guaiacol, eugenol, and vanillin (Chatonnet *et al*., 1997; Spillman *et al*., 1997; Kennison *et al*., 2008). At low levels, these compounds add complexity to the wine aroma (Francis & Newton 2005), but higher levels are undesirable and usually considered to cause an off-flavour or taint (Boidron et al. 1988, Kennison et al. 2007). VPs in wine may come from a number of sources including yeast fermentation (Romano *et al.*, 2009; Weiss, 2014) and wood maturation (Boidron *et al.*, 1988; Prida & Chatonnet, 2010). Sefton (1998), and Wirth *et al.* (2001) reported that guaiacol and 4-MG occurred naturally in the fruit and leaves of Shiraz, Merlot and Muscat of Alexandria. Ristic et al. (2015), showed that control Shiraz wines had higher levels of guaiacol compared to those of Merlot.

Guaiacol and 4-MG are derived from oak lignin degradation products and are therefore commonly found in wines that have been aged in oak barrels (Pollnitz et al. 2000, Singh, et al., 2011). Usually extraction from oakwood by wine is in the range of 10–100 µg/L and 1–20 µg/L, for guaiacol and 4-MG respectively (Pollnitz, *et al*., 2004). The cresols, as well as 3, 4-dimethylphenol (3,4-DMP), guaiacol and 4-EP have also been linked to lignin pyrolysis during the toasting of oak barrels (Etievant, 1981; Cadahía *et al.*, 2003; Fernandez de Simon *et al.*, 2008).

Volatile phenols (can also be produced in red wine from the bioconversion of hydroxycinnamic acids in grapes (Fugelsang & Edwards, 2007), notably by yeast of the *Brettanomyces* genus, (Chatonnet *et al* 1992; Romano *et al*. 2009). If produced in large enough concentrations, this may lead to the so-called "brett" taint. VPs responsible for this taint include 4-EP, 4-EG and 4-ethylcatechol (4-EC), which are reduced from their respective vinylphenol derivatives (Malfeito-Ferreira *et al.,* 2009).

Other sources of VPs in food products may come from motor exhausts and residential emissions in highly populated areas (Allen, 2010). Industrial hazes (Goldammer et al., 2009) have also been noted as sources with many aromatic compounds identified in air pollution.

Although VPs, as noted, may derive from a number of sources, a lot of research in recent years concerning VPs has been centered on smoke taint (Krstic *et al.*, 2015), which is the off-odour that results from exposure of grapes to bushfire smoke. Work related to smoke taint in wine follows a logical progression of discovery over the years since it started, with Australian researchers leading the field. The effects of two of the key compounds associated with smoke-taint in wine (guaiacol and 4-MG) have been well described (Kennison *et al.*, 2011; De Vries, Buica, *et al.*, 2016), and include 'burnt', 'smoky' and 'ashy' aromas and flavours. Studies focusing on the chemical measurement of concentrations of these and other VPs (including the cresols, syringol and 4-methyl syringol) in smoke tainted grapes and wines and VP glycosides (Hayasaka *et al.*, 2010; Wilkinson *et al.*, 2011; Kelly, *et al.,* 2014; Noestheden, et al., 2018) are extensive and ongoing. Kennison *et al.,* (2008, 2009, 2011) demonstrated a direct association between grapes exposed to smoke during the growing season and the presence of VPs in Merlot juice following grapevine exposure to smoke. Wilkinson *et al.,* (2011) compared methods of analysis of VPs and their glycoconjugates in grapes and wine. Kelly *et al.*, (2012) assessed exposure of grapes to smoke of vegetation with varying lignin composition and the accretion of lignin-derived putative smoke taint compounds in wine. Fudge *et al.*, (2011) investigated the effect of reverse osmosis and solid phase adsorption in ameliorating smoke taint in wine, and Kelly *et al.*, (2014) examined extraction effects of winemaking practices on VPs, as well as sensory profiles associated with them (Kelly *et al.*, 2014; Kelly & Zerihun, 2015). Although it was shown that juice from smoke-exposed grapes seldom contains significant levels of VPs, by the time fermentation is over, free VP levels rise tenfold, and in the case of guaiacol, hundredfold (Kennison *et al.,*2008) due to the activity of yeast and bacterial enzymes active during fermentation. Krstic *et al.*, (2015) reviewed smoke taint derived VPs and their glycosidic metabolites in grapes and vines as biomarkers for smoke exposure and their role in the sensory perception of smoke taint. Ristic *et al.*, (2017) investigated the effect of bottle aging on the release of VPs during maturation. Hayasaka, (2010) also assessed the impact of smoke exposure in grapes, quantifying VP glycosides.

As forest fires close to vineyards are becoming more frequent (Fried *et al.*, 2004; Parker *et al.*, 2012; Wolf, 2018), wine producers need to be aware at what concentration smoke-related compounds affect the sensory properties of a particular wine. Smoke particles are known to travel many kilometres from their source and settle over large areas (Goldammer *et al.*, 2009) which may affect crops including grapevines. Thus the role of VPs in smoke taint in wine, as well as other agricultural crops is a primary focus as this phenomenon has a severe economic impact on producers, and is unlikely to decrease, given the effects of global warming (Strydom & Savage, 2016).

The effects of VPs on the aroma profile of red wine have long been known. They have been linked to a number of other off-odour issues on a continuum of aromas in red wine including the previously mentioned 'brettiness' and 'smoke taint'. Chatonnet *et al.*, (1992), defined the effect of volatile phenols produced by microorganisms in wine during aging as an off-flavour known as '*phenolé*' or 'phenolic', ('disagreeable animal, stable') odour. In a large study characterising the odours of 30 alkylated volatile phenols in air, Czerny *et al.*, (2011) noted that phenol itself was 'ink-like' in aroma, and mono- alkylated phenols were generally described as 'medicinal', 'ink-like', 'leather', 'horse', and smoky'. The VPs 4-EG and 4-EP are associated with 'medicinal', 'horse/leather' and 'barnyard' characters, and although they can impart smoky aromas/flavours, they are not considered markers of smoke taint. Dimethylated phenols have been described as 'burnt, with trimethylated phenols distinguishable as having 'smoky and 'rubber' characteristics in addition to the previously listed attributes. The authors observed that the phenolic hydroxyl group was important factor for the odour characteristics of this class of compounds, as the corresponding toluenes were almost odourless. Aroma descriptors have been established in water for a number of the volatile phenols (Czerny *et al.*, 2008), including guaiacol, the furfurals and 4-ethyl and vinyl phenol. Some of these compounds are a normal part of a complex wine aroma profile when present at low levels, but can cause problems at higher concentrations, because they suppress fruitiness (Atanasova *et al.*, 2004; Ferreira, 2012a) and impart smoky, charred or burnt aromas (Kennison, 2013). Guaiacol forms part of the 'woody' family of descriptors (Noble *et al*., 1987), and exhibits 'burnt', 'smoky' and 'sweet-burnt' characteristics. At low levels, it is not known to cause off-flavours in red wine. Parker *et al*., (2012) however, did show that guaiacol, 4-methylsyringol (4-MS), 4-MG, phenol and *o*- and *m*-cresol were positively correlated with both smoke aroma and ashy aftertaste. Many of the VPs in their study were positively associated with a 'medicinal' attribute, with syringol, *o*-cresol and 4-methylsyringol being large contributors to smoke taint. Smoke-related sensory attributes were well predicted by the volatile phenol data in their study.

In later work, Parker *et al*., (2013) described the aroma of *o*-cresol as 'medicinal' and 'smoky'. Boidron *et al.*, (1988) had also described it as 'tar' (bitumen)-smelling, in a significant study of the aroma compounds found in wine and derived from oak, which was substantiated by Panzeri (2013) and De Vries, Buica, *et al.,* (2016). Their study also indicated that volatile phenols and their glycosides produces an undesirable smoke flavour in affected wines. Descriptors for 4-EP, the compound most closely associated with 'brettiness' in wine, include 'horsey', 'leather', 'Band-aid' or 'Elastoplast™' (Oelofse *et al.,* 2009), medicinal', 'smoky' (Kennison *et al.*, 2008, 2011) 'faecal' and 'horse-stable' (Czerny *et al.*, 2011). Aroma attributes used to describe wines made from grapes exposed to smoke also include 'burnt rubber', leather', 'disinfectant' and 'smoked meat' (Kennison *et al.*, 2009).

1.2 3-Isobutyl-2-methoxypyrazine (IBMP)

Consumers rarely appreciate red wines with pronounced herbaceous flavours (Mozzon *et al*, 2016). If 'greenness' is present, products are described as possessing immature character, lacking refinement and elegance, and having reduced fruit intensity. The 'herbaceous' and 'vegetative' character has also been shown to detrimentally impact palate structure and give a 'green tannin' mouthfeel, but evidence is anecdotal and there is little in the primary literature to back this up. Parr *et al.*, (2007) noted that even with Sauvignon Blanc, a cultivar renowned for 'greenness', not all 'green' characters (notably 'leafy', 'stalky', or 'vegetal') are positively correlated with typicality or good varietal definition. Although it is well known that methoxypyrazines, and specifically 3-isobutyl-2- methoxypyrazine, are responsible for 'bell pepper' and 'herbaceous' notes in wine (Allen *et al.*, 1996; Marais & Swart, 1999), little has been written concerning the interaction of these compounds with other taint compounds in wine. Lapalus *et al.*, (2016) characterised the sensory attributes and chemical composition of thirteen selected South African Cabernet Sauvignon wines and found that supra-threshold concentrations of IBMP correlated with 'fresh green' and 'gherkins/jalapeno' attributes. However, 'eucalyptus/mint', and 'bay leaves', were poorly explained, by the compositional data which warranted further investigation. Mozzon *et al.*, (2016) emphasised the critical importance of understanding the origins and causes of these 'green' off-flavour characters order to reduce or avoid these olfactory qualities in wines.

1.3 2,4,6-Trichloroanisole (TCA)

In wine, 2,4,6-trichloroanisole (TCA) is known to be the main compound responsible for 'cork taint' (Buser *et al.*, 1982), described as having a 'musty' (Griffiths, 1974), 'mouldy' and 'damp cardboard' odour (Cravero *et al.*, 2015). TCA is a product of the fungal activity, is an easily recognised compound because of its distinct odour and low sensorial threshold (Sefton & Simpson, 2005). The off-flavour has considerable economic impact due to the rejection of wine by consumers (Van Eeden, 2009), even if the taint is only present at very low levels. Tempere *et al.*, (2017) noted that amongst wine defects, TCA has a specific impact on wine perception: in addition to giving to the wine an unpleasant odour, it has a strong masking effect on fruity notes. Despite the 'notoriety' of the compound in wine and its low threshold levels, studies involving TCA focus on the measurement of the compound in tainted wines (Buser *et al.*, 1982; Boidron *et al.*, 1988; Pollnitz *et al.*, 1996; Prescott *et al.*, 2005) and corks (Pollnitz *et al.*, 1996; Taylor *et al.*, 2000; Alvarez-

Rodríguez *et al.*, 2002), but it is difficult to find studies where its sensory effects are formally tested in combination with other compounds or in different matrices.

2. Factors affecting the olfactory perception of taint compounds in red wine

2.1 Odour Detection Thresholds (ODTs)

One of the parameters used when discussing odours, particularly in wine research, is the "Odour Detection Threshold" (or ODT). A detection threshold is defined as the lowest concentration of a substance in a medium at which a stimulus is detected, but not necessarily recognised (Lawless & Heymann, 1999). In wine evaluation, detection thresholds are usually olfactory or taste related, but use of the tactile sense is also important in mouthfeel, such as astringency and 'spritz' or mousse in sparkling wines (Le Barbé, 2003). The concentrations of volatiles reaching olfactory receptors can range from levels well below threshold (infra-threshold), where no aroma is perceived, to levels well above threshold (supra-threshold), where the smell is obvious. Wine researchers recognise the ODT as the lowest concentration of a compound at which individuals can reliably perceive a difference in odour stimulus, with an operational definition of 50% performance above chance (Perry & Hayes, 2016).

Sensory thresholds, but are based on the minimum amount of compound added to a clean control that elicits, from a human subject, a recognition of difference in sensory quality between sample and control (Lawless & Heymann, 1999). Usually, ODT determinations in solutions are based on the use of a dilution series which presents the odour compound diluted at precise ratios to a panel of assessors. The most commonly used tests for the determination of detection thresholds are duo-trio, triangle, and R-index difference tests (Lawless & Heymann, 2010). A sufficient ODT determination would require hundreds of comparisons with a control, but due to the unique nature of the product, sensory studies on wine aroma are very specific to particular cultivars and styles, and frequently suffer from a lack of repeatability. Lawless & Heymann, (2010) have stated that threshold studies relying on the "50% perceive a difference test" are arbitrary and empirical, and as such there is no scientific basis for such a test. Some authors have gone as far as to suggest it may be futile to invest time and money in threshold studies (Meilgaard *et al.*, 2016). However, researchers do need some sort of measure against which to quantify effects of treatments, and the threshold approach, using as many subjects as possible, in the matrix in which a study is to be conducted, has been shown to be simple and reliable and is still the best strategy available (Meilgaard *et al.*, 2016).

Another parameter used frequently in wine evaluation is the "Odour Activity Value" (OAV). In the literature, the stimulus presented by a specific odorant is often expressed in terms of an OAV, which is defined as the ratio between the concentration of the compound in the wine and the detection threshold for that compound (Prida & Chatonnet, 2010). OAVs seem to be a relatively simple way for determining the potential sensory impact of chemical compounds, as a value >1 is considered "odour-active" (Prida & Chatonnet, 2010; Ferreira, 2012), but they often do not correlate with intensity of aroma perception, and they cannot predict how various compounds are perceived at different concentrations, and in different matrices. If OAVs are assigned and applied arbitrarily across different matrices, they will rely solely on the physicochemical parameters of the solution, and not take into account the other factors involved in sensory perception (Audouin *et al.*, 2001). It is unlikely that they will accurately reflect the complexity of the effects of the solution on sensory perception of target aroma compounds.

2.1.1 ODTs for Volatile Phenols

Guaiacol and 4-MG exhibited the lowest odour detection thresholds 75 mg/L and 65 mg/L, respectively, of all the phenols tested in a red wine matrix by Boidron *et al.*, (1988). Eisele & Semon (2006) reported a very low 'best estimate threshold' (ISO: 13301, 2002) for guaiacol of 0.48 µg/L in water, but also noted that there was about a 500-fold range in guaiacol detection between panellists, with some individuals exhibiting a BET as low as 10 ng/L. Parker *et al.*, (2012) reported a Best Estimate Threshold (BET) for guaiacol detection in red wine as 23 ± 0.8 µg/L and 27 ± 0.6 µg/L for guaiacol flavour (in mouth). Simpson *et al.*, (1986) reported a lower ODT of 20 µg/L for guaiacol in white wine.

It is difficult to find any sensory information in the primary literature regarding ODTs for the cresols. There has been some work done in whisky, but the only current wine-related references concern the appearance of cresols in wine after smoke-events in the vineyard. Boidron *et al.*, (1988) gave the 'bitumen'-smelling *o*-cresol a sensory threshold of 800 µg/L BET which seems extraordinarily high, but the study was looking specifically at wooded wines, and heavily toasted wood is very likely to have elevated levels of pyrolysis products. The panel would also have been exposed to sixteen aromatic wood-derived compounds (ten of which were VPs), and possibly could have suffered from sensory fatigue or saturation. Parker *et al.*, (2012) reported a BET for *o*-cresol odour, through a formal sensory process with 22 assessors, as 31 µg/L in model wine (alcohol solution), and 62 ± 0.8 µg/L, for *o*-cresol in 'base red' wine. This group also determined the thresholds for other cresols, and found *m*-cresol had the lowest BET at 20 µg/L.

The third VP, 4-EP, is associated with 'brettiness' and 'medicinal, Band-aid' smells (Oelofse *et al.,* 2009), as well as 'leather/ horse' and 'bacon/meatiness' (Boidron *et al.*, 1988; Chatonnet *et al.*, 1992; Krstic *et al.*, 2015). It has complex effects in wine. The detection threshold determined by Chatonnet *et al.*, (1992) is commonly cited, which is 130 µg/L in water, 440 µg/L in model wine solution and 605 µg/L in red wine. In Chatonnet's study, which focuses on the microbial origin of phenols, thresholds were defined as the minimum concentration below which 50% of 70 panellists failed to distinguish the sample from a control, as described by Boidron *et al.,* (1988) in their study of effects of wood. For each substance studied and its mixture, the perception threshold of each taster was determined in a standard red wine (this was called a “Recovery Threshold”) by a triangular directional test. Curtin *et al.,* (2008) determined the ODTs for 4-EP, 4-ethylguaiacol (4EG) and 4-ethylcatechol (4EC) in a study on ‘brett’ character in red wine. They realised the importance of matrix effect on ODTs and used a ‘neutral’ wine, an ‘oaky’ wine and a ‘green’ wine. Unsurprisingly, given the more complex aroma of the ‘oaky’ wine, the ODTs were found to be significantly higher in ‘oaky’ wines, and slightly higher in ‘green’ wines, than in ‘neutral’ wines. The ODT of 4-EP was 368 µg/L in the neutral wine, 425 µg/L in the ‘green’ matrix and 569 µg/L in the ‘oaky’ wine. No details of the method used for the determination of detection thresholds were included in the publication. In a later study, Botha, (2010) determined the ODT of 4-EP to be 201 µg/L (median value) and 221 µg/L using the ASTM method in Pinotage. According to Escudero *et al.,* (2007), 4-EP falls in the same semantic category as woody odorants, and in wooded wines, the wood character may therefore mask, or incorporate, the aroma character of 4-EP, making it more difficult to detect as a specific character (leather/horse/ medicinal). This is in agreement with the findings of Curtin *et al.*, (2008) and seems to explain the very high threshold found by Chatonnet *et al.*, (1992). Botha (2010) ascribed the lower detection threshold in their study to the fact that they used unwooded Pinotage.

2.1.2 ODTs for other compounds in the study (IBMP and TCA)

IBMP is often found in wine at concentrations that are above its odour threshold (Allen *et al.*, 1996; Roujou De Boubée *et al.*, 2000). Low levels contribute to the aromatic complexity of red wines (Roujou De Boubée *et al.*, 2000), but higher levels are perceived as ”dustiness”, “greenness”, “herbaceousness” and are associated with a lack of ripeness (Suklje *et al.*, 2012). Higher levels are seen as detrimental to red wine quality (Allen & Lacey, 1996; Roujou De Boubée *et al.*, 2000); Roujou de Boubée et al., 2000). It is difficult to find information on the determination of odour thresholds for methoxypyrazines in the literature. They are strong-smelling compounds, do not break down easily, and pose challenges in analysis (Albert *et al.*, 2013). A further challenge to evaluation is posed by the fact that people have very different sensitivities to methoxypyrazines: Shibamoto (1986) determined odour thresholds of 46 pyrazines in water using a panel of seven males, and found that compounds were detected across a very wide range from 0.01 µg/L to 6.00 mg/L. The author did not, however, test 2-isobutyl-3-methoxypyrazine. Marais & Swart, (1999) established the effect of the lowest levels of addition of IBMP (2 ng/L and 4 ng/L) to Sauvignon Blanc wine as ‘dustiness’ and ‘grassiness’ respectively. This was not conducted as a full, formal sensory determination, as only eight judges were used. Effect in red wine was not tested. French studies of Cabernet Sauvignon and Cabernet Franc production in 1991 and 1992 in Bordeaux and the Loire showed that IBMP was the main contributor to vegetal aroma (Roujou De Boubée *et al.*, 2000). The threshold of detection in wines was determined by comparing IBMP concentrations of 50 red Bordeaux and Loire wines from different vintages and grape varieties, with the intensity of the ‘bell pepper’ character as perceived on tasting. Through this, the threshold value, which seems to be rather a recognition threshold in the context of that study and not a perception threshold, was estimated to be 15 ng/L. This estimate of threshold value does not seem to have been confirmed by any further formal sensory studies. A study by Alberts *et al* (2013), used odour detection thresholds from a 1996 study by Allen & Lacey, to estimate the flavour contribution of IBMP, and stated that a ‘combined concentration’ (assuming an additive effect /positive interaction) of 4 ng/L to 8 ng/L will make the ‘herbaceous’ or ‘vegetative’ aroma evident in white wine, while the ‘optimum concentration for Sauvignon Blanc wine has been described as 8 ng/L to 15 ng/L’. Allen & Lacey (1996) argued that sensory perception at concentrations > 30 ng/L are considered overpowering and out of balance.

Lapalus *et al.*, (2016) investigated the relationship between the volatile composition and sensory properties in 13 mono-varietal Cabernet Sauvignon wines produced in South Africa. The wines were selected to represent a broad range of fruity and herbaceous sensory attributes and were assessed by descriptive analysis (DA). The statistical treatment by multiple factor analysis (MFA) of both compositional data and sensory data showed that amongst other compounds, levels of IBMP predicted some but not all of the aroma attributes used to describe the selected wines. Mozzon *et al.*, (2016) noted that a deeper understanding of the origin of these herbaceous characters and, perhaps more importantly, a prediction for their effect on the finished wine is needed.

It is difficult to find information on formal sensory detection threshold determinations of TCA, but Young *et al.*, (1996), determined the taste and odour threshold concentrations in water for various anisoles, using dilutions series and panels comprising a minimum of six specially selected and trained assessors. They found the geometric mean odour threshold concentration for six panellists for TCA in water to be 0.9 ng/L, and lowest concentration at which ‘an odour’ was detected to be 0.08 ng/L. Tempere, *et al.,* (2013) confirmed that distribution of individual detection thresholds for TCA by panellists in Merlot Noir wine covered a wide concentration range. Studies seem to confirm this, as ODTs range from 0.03 to 1–2 ng/L in water (Griffiths, 1974) and up to 4 ng/L in a white wine for trained assessors (Sefton & Simpson, 2005). Tempere *et al.*, (2017), determined a level of 0.13 ng/L TCA in red wine was considered ”low” and 5 ng/L was considered “high” based on concentrations frequently found in contaminated red wines. Mazzoleni & Maggi (2007) also noted that the detection threshold of TCA varies widely depending on the organoleptic characteristics of the matrix, and the person perceiving it. These authors tested detection of TCA with 14 panellists in seven different red wine cultivars and found that the compound was perceived by >50% of panellists at either 10 or 15 ng/L depending on the matrix. Prescott *et al.*, (2005) established a much lower ‘consumer rejection threshold’ of TCA in Chardonnay, of 3.1 ng/L and 3.7 ng/L depending on panel.

Takeuchi *et al.*, (2013) investigated off-flavour substances generated naturally in foods/beverages and showed that TCA was detected in a wide variety of foods and beverages surveyed for odour losses. These authors observed that even at very low levels, TCA inhibited ciliary transduction channels in single olfactory receptor cells, and showed slow kinetics in its inhibitory effect on plasma membranes. The specificity and efficacy of the masking effect of TCA was tested at infra (sub)- and supra-threshold concentrations. Tempere *et al.* (2017) used a simplified model of a binary mixture for in-depth analysis of the masking effect of TCA. Their results provided experimental confirmation that constituents in non-perceptible concentrations of TCA influence the perceived quality of mixtures of odorous compounds, and confirmed the work done by Takeuchi *et al.*, (2013) of interactions taking place at receptor level.

2.2 Matrix effects on olfactory perception of taint compounds

More than thousand volatiles have been identified in wine. To be perceived, these aroma compounds need to volatilise from the matrix into the headspace of a glass and reach the olfactory epithelium of the taster (Cameleyre *et al.*, 2018). From a physicochemical point of view, this release depends on the composition of the matrix, as volatility can be affected by the ionic strength of the solution as compounds can form weak physical bonds with the solvent, based on polarity. If polarity changes (for example, with % ethanol), the volatility of compounds changes which then affects the partition coefficient of the compound between liquid and gas phase (Vilanova & Oliveira, 2012). Matrix effects on the perception of aroma of solutions are thus important. The lack of repeatability in studies of wine aroma is unsurprising, as odour thresholds used by wine researchers are often previously established in matrices that are not appropriate to the study undertaken. Perry & Hayes (2016) emphasised the strong need to carefully consider the composition of the delivery matrix when determining and comparing threshold estimates across studies. For example, a researcher might use the ODT for a volatile phenol that has been ‘confirmed’ in water or model wine, for a study in red wine and find that perceptual effects were not repeatable. This is confirmed by the work of other authors where thresholds determined in wine are usually significantly different to those determined in model solution or water (Le Berre *et al.*, 2007; Botha, 2010). Even within a broad category (white or red), the wine-style and choice of cultivar may alter detection thresholds (Martineau *et al.*, 1995; Jackson, 2014), but evidence from formal studies looking at this aspect is hard to find. In wine, even something as obvious as % v/v alcohol can affect perception of thresholds of odour compounds, but surprisingly few studies exist to examine this aspect. As alcohol percentage increases, volatility of a number of compounds decreases as most aroma compounds are fairly hydrophobic, and so if more alcohol is present, they will stay in solution (Goldner *et al.*, 2009). Thus, wines with a higher %v/v alcohol may be less aromatic, or totally different in aroma from those with lower alcohol concentrations. Petrozziello *et al.*, (2014) found that polyphenols and ethanol have a significant influence on the olfactory perception of ‘brett’-tainted wines, seeming to reduce the volatility of 4-ethylphenol.

Another factor affecting olfactory perception of odours is the concentration of other odorants in the matrix. Wilson *et al.*, (2018) showed that the perception of thiols was affected by the volatile and non-volatile wine matrix. Tempere *et al.*, (2016) observed that both supra and sub-threshold concentrations of off-flavour components may change the perception of odorous mixtures. They looked at the impact of infra- and supra-threshold concentrations of ethylphenols on wine, and found that both sub- and supraliminal concentrations of off-flavours not only change the "hedonic valence" (positive or negative character) of the perception, and have a masking effect on fruity notes. Kaeppler & Mueller, (2013) noted that in olfaction, the quality and intensity of a compound interact considerably and a shift in one dimension is often accompanied by a shift in another dimension: to quote: "Whereas a colour keeps its basic quality (blue) with increasing or decreasing intensity (light blue, dark blue), odours often change their quality with higher or lower concentrations".

Robinson *et al*., 2009 showed that glucose increased the concentration of volatiles in the headspace of a wine, whereas increasing ethanol concentration was negatively correlated with headspace partitioning of volatiles. The reduction of headspace concentration of volatiles suggests that higher ethanol concentrations may suppress 'fruity' attributes in wine. This ties in with work by Pineau & Barbe (2009) who carried out sensory reconstitution tests and established that very small variations in the concentrations of certain ethyl esters were perceivable in de-aromatised red wine and affected the red- and black-berry aromas. In fact, an increase of as little as 1.3% of the concentration of ethyl 2-methyl propanoate modified the assessors' aromatic perception of the matrix. In model wine, recognition tests were all significant at $p<0.001$, whereas, in de-aromatised red wine, test matrices with higher than average concentrations of ethyl hexanoate, ethyl octanoate, or ethyl 3-hydroxybutanoate were not recognised. Thus, the authors concluded that model wines do not provide a realistic scenario for threshold studies for wine. The argument for using de-aromatised red wine to study the aromatic impact of volatiles was supported. In fact, odour thresholds in any matrix other than the study matrix may be irrelevant, as suggested by Meilgaard *et al.*, (2016). Ideally, a researcher should work out each individual ODT for each individual matrix, and stay in that specific matrix for the entire duration of a study. Data generated without establishing the ODT in the study matrix are meaningless, as the threshold of odour detection will be different in every cultivar and style. As Heymann (2018, personal communication) observed, "There is no way to make predictions about anything as it is all matrix dependent".

2.3 The 'judge effect' (differences between panellists) in olfactory perception

Another important issue affecting differences in the perception of odours in solution is that brought about by the 'judge-effect': *i.e.* differences between individuals in terms of their perceptual ability, experience and context.

Tempere *et al.*, (2012) noted that studies have shown considerable variation in chemosensory human capacities. Sensitivity to odours is variable among different individuals, so panellists in a testing situation could assign different odour concentrations to the same sample (Brattoli *et al.*, 2011). Not only does human olfactory perceptive ability differ enormously between individuals, but also the hedonic response varies. Keller *et al.,* (2007) reported large perceptual variations in the intensity and pleasantness of androstenone, an odorous steroid. It was variously perceived by different individuals as offensive ('sweaty', 'urinous'), pleasant ('sweet', 'floral') or 'odourless'. In addition to their own physiology, many factors are known to affect panellists, including the time of day, illness (Brattoli *et al.*, 2011), ambient temperature and odour, sensory fatigue (Ferdenzi *et al.*, 2014), and the effects of other compounds present in the wine such as alcohol (Goldner *et al.*, 2009). Gender is also known to have an effect on ability to detect odours, with women being recognised as generally more sensitive to a wider range of odours in wine (Wurz *et al.*, 2017). Tempere, *et al.,* (2016) noted that even wine tasting experts can show high olfactory detection thresholds for key compounds of wine, which is not ideal when wine quality depends on fault-detection at low levels, and adapted training for professionals in the wine industry may be appropriate.

Several studies have shown that odour quality perception is shaped by experience and have illustrated in cross-cultural comparisons (Pangborn *et al.*, 1988; Chrea *et al.*, 2004; Wilson, 2005), and across a person's life span, physiological changes and experience may alter olfactory perception (Barkat *et al.*, 2012). Well-known odours are usually rated as more pleasant which suggests that humans prefer the smells they frequently experience (Distel *et al.*, 1999), confirming the experience-dependency of odour quality judgments (Kaeppler & Mueller, 2013). Thus, people at comparable ages, with similar cultural backgrounds, and without physiological issues, may have similar olfactory perceptions. Experience has also been shown to influence quality perception by providing background on the identity, function, or effect of an odour. Barkat *et al.*, 2012 outlined that there seems to be conflicting evidence for whether experience and training could have an impact on olfactory processing and perception, but that their study revealed differences in the perception of mixtures between naïve subjects and experts. These authors supported the idea that olfactory expertise can modify perception of a mixture and distinguish elements from the configuration. Expert subjects were more accurate in identifying individual odours due to more sharply defined internal reference as a result of exposure to odour reference standards (Barkat *et al.*, 2012). It has been shown that the training has a positive effect on the sensitivity of panellists for particular odours (Tempere *et al.*, 2012), and that culturally acquired experience (for example, cooking specific foods) affects the evaluation of familiar versus unfamiliar odours rather than perceptual processes in general (Ayabe-Kanamura *et al.*, 1998). Panellists with the same experience level tend to evaluate consistently but experts use additional and specific descriptors to verbalise their perceptions (Lawless & Heymann, 2010) which could be ascribed to enhanced perceptual skills (Parr *et al.*, 2011). Training might enhance both perceptual and verbal skills, and enrich olfactory terminology. Laymen usually have major difficulties in naming even familiar odours correctly and identification rates rarely exceed 50% (Cain *et al.*, 1998).

Odours are also powerful cues for autobiographical memories (Kaeppler & Mueller, 2013). Thus, when people cannot identify an odour, they will associate it with places and situations (“the beach”), activities (“cleaning,” “gardening”), effects (“peaceful”), or—on the most basic level—hedonic ratings (“nice” or “horrible”). Chrea *et al.*, (2004) argued that odours are only arranged semantically when verbal or visual identifiers are available, so to facilitate satisfactory communication despite inadequacy of language, odour professionals have acquired cognitive categories and established professional terminologies (lexicons) that allow them to allocate odours to discrete conceptual categories. An example of this would be the ‘Aroma Wheel’ conceptualised by Noble *et al.*, 1987. It is a matter of scientific debate whether odour categories are innate and thus universal, or learned. A mental representation of an odour, also called an “Odour-object” (Wilson & Sullivan, 2011) will be shaped by various interactions between its characteristics as well as the impact of physiology, knowledge, culture. Unquestionably, the odour classes applied by odour professionals are acquired, with various findings indicating that experience yields odour arrangements that are different from the systems used by non-professionals which include the “pleasantness” factor. When attribute lists devised by experts are used by laymen, terms are misunderstood and used differently by the untrained subjects (Lawless, 1999; Kaeppler & Mueller, 2013). When sensory panellists agree on their perceptions in an ‘alignment’ exercise, this may still overrate or underemphasise certain quality aspects, depending on study requirements, but this aspect of DA has not been very well researched. Different approaches to data analysis will also yield results that might not accurately reflect the perceptions of laymen when the data are interpreted by researchers or specialists, a qualitative data management issue that cross-cuts disciplines.

Elucidating meaningful links between even simple chemical structures and perceived odours from sensory data, the so-called SORs or ‘structure-odour-relationships’ (Kaeppler & Mueller, 2013) have not met with complete success. Modern computational approaches and access to thousands of physicochemical configurations and odour attributes have provided a few basic conclusions, but a lot of questions remain unanswered (Sell, 2006; de March *et al.*, 2015; Keller *et al.*, 2017). The olfactory perception space is acknowledged to be weak and ‘highly dimensional’ (Kaeppler & Mueller, 2013), nomenclature is often arbitrary, and odour classes overlap. The reliability of both perception and verbal expression are questionable and olfactory perceptions are not necessarily stable over time (Wilson & Sullivan, 2011). It is well-recognised that different people do not perceive identical odorants in the same way, and do not verbalise their olfactory perceptions consistently, but that this has not been well-researched. Questions also remain around SORs of broad classes of molecules, the role of functional groups in the perception of odour, characterisation of single compounds at various levels and the perception of odour-interactions for simple and more complex mixtures.

2.4 Interaction effects

A much more complex aspect of wine aroma than the partitioning between gas and liquid phases is the perceptual interaction between various aromatic compounds, including synergistic (hyperadditive) effects (Lytra *et al.*, 2012, 2013) and masking effects. Research has shown that aroma compounds in wine such as thiols and terpenes 'interact' (Atanasova *et al.*, 2004; Ferreira *et al.*, 2016; Wilson *et al.*, 2018) or (to be more precise) manifest different olfactory perceptual characteristics when compounds are present as mixtures in solution compared to solutions containing single compounds. In the case of wine, these perceptual effects are interpreted as changes to the aroma profile, which may lead to increased complexity, or decreases in typicality of cultivar or style. It may be the case that certain compounds, while not present individually at sufficient intensity to cause a quality issue, can 'interact' when present in combination, leading to off-odours (Panzeri, 2013).

2.4.1 The nature of olfactory perceptual interactions

As most real-world odours are complex mixtures of distinct components, olfactory systems will adopt different strategies to contend with this complexity. In elemental processing, odour perception is derived from the sum of its parts; in configural processing, the parts are integrated into unique perceptual wholes (Howard & Gottfried, 2014). Mammalian odour receptors (OR) are able to detect and distinguish between thousands of odours, in a combinatorial response to odorant molecules. A single aroma can elicit response from multiple receptors, or a single receptor could respond to multiple odorants (Wilson & Stevenson, 2010). An odorant may have a unique combination of responses from several receptors, which endows the olfactory system with powerful discriminatory ability. The mechanisms by which the olfactory system functions are starting to be elucidated in physiological studies in insects like fruit flies (Christiaens *et al.*, 2014) and mammals like mice (Haddad *et al.*, 2010; Wilson & Sullivan, 2011). Chemists have also been trying to link olfactory properties with chemical structures (Czerny *et al.*, 2011; de March *et al.*, 2015) and geneticists have been attempting to pinpoint genes associated with olfaction (Wooding, 2013; Sell, 2014). Different types of interactions can occur at the peripheral level, depending on the odorant concentration ratios, which affect a mixture's perceptual properties. Wilson & Stevenson (2010) hypothesised that perceptual grouping and pattern recognition abilities of the piriform cortex place an upper limit of three components on odour mixture analysis by humans. Beyond that limit, individual component analysis becomes faulty and odorant mixtures are processed as a 'single perceptual gestalt'. Although odorant mixture interactions can occur even with novel odorants, experience can shape cortical mixture processing. Also, when a mixture is familiar, cortical neurons treat the mixture as a unique object, different from its components, whereas without experience, cortical neurons treat mixtures and their components as more similar. Studies in olfactory perception have also shown the importance of peripheral interactions and past experience (Barkat *et al.*, 2012) in the coding of complex odorant mixtures. Howard & Gottfried (2014) emphasised that the rules that govern the involvement of either elemental or configural processes during odour perception were poorly investigated. There is also little understanding of peripheral coding of odorants' mixtures even though the properties of individual components may be known (Thomas-Danguin *et al.*, 2014). The physiological origin of olfactory interactions specific to wine has been addressed in very few studies (Chaput *et al.*, 2012; Silva Teixeira *et al.*, 2015), and there are still a great number of unexplained and unexplored issues around wine-related olfactory perception and learning.

One possible explanation of this lack of investigation, especially in humans, could be the difficulty quantifying odour quality (Barkat *et al.*, 2012), for the reasons given in Section 4 above. Barkat *et al.*, (2012) noted that the choice of sensory method is a critical step in investigating blending processes in odour mixtures. This is especially important when perceptual odour blending and interactions may affect the mixture's odour quality. These authors recommended that a detailed aroma-profiling task involving both single component descriptors and a main character descriptor should be undertaken if odour blends are to be described. However, a limitation of such a procedure would be that the panellists are engaged in an analytical perceptual processing strategy, which could decrease synthetic processing and consequently the blending effect (Le Berre *et al.*, 2007). Aroma-profiling tasks that require odour references for each descriptor that are presented at the beginning of panel sessions might also modulate the latter perception and evaluation of blending mixtures (Barkat *et al.*, 2012).

Perception of odour mixtures, such as those present in wine, is far more complicated due to interactions arising from the complex chemical signal encoding and processing within the olfactory system (Thomas-Danguin *et al.*, 2014). The variety of sensory perceptions observed when presented with mixtures of odorants could be the result of both qualitative (odour quality) and quantitative (odour intensity) perceptual interactions between odorants (Laing *et al.*, 1984, 1994). The theory of 'odour-object' encoding underpins the neurophysiological processes involved in extracting only relevant information from complex chemical mixtures in the environment (Wilson & Stevenson, 2010; Thomas-Danguin *et al.*, 2014). Interactions occurring at the peripheral level of the olfactory system play an important role in processing odorant mixtures and triggering the coding (Laing *et al.*, 1984). Initially, odorants are sampled by a large number of ORs located in the cilia of olfactory sensory neurons/cells (OSNs). Each OSN/OR usually responds to a variety of odorants so that the identity of a molecule is encoded by the combination of ORs/OSNs that recognise it (Thomas-Danguin *et al.*, 2014). The overlapping response profiles of OSNs and subsequent encoding is under cognitive control and learning will shape perceptions, and continued exposure will lead to the experience of mixtures as odour-objects or specific odour configurations

(Thomas-Danguin *et al.*, 2014). An efficient memory-based olfactory system would learn which features should be grouped (associated) together to form a single olfactory odour-object (e.g. 'smoke') despite the fact that this odour is composed of hundreds of components (Wilson, 2005), and very complex stimuli would be simplified, vastly extending the tuning range of the olfactory system. The perceptual configuration of mixtures into simpler odour-objects would thus improve an organism's ability to extract information from the environment (Wilson & Stevenson, 2010), and cognitive processes should decrease the chemical complexity of the environment by building experience-dependent perceptual associations (Wilson & Stevenson, 2010).

Studies in different species have compared the responses of OSNs to binary mixtures and their components (Thomas-Danguin *et al.*, 2014), and data modelling (Munch *et al.*, 2013) suggests that both competitive and non-competitive interactions occur at receptor level. Thomas-Danguin *et al.*, (2014) note that there is competitive interaction when two molecules bind to the same receptor binding site which might involve agonist (molecules that activate the receptor) odorants, or agonist/ antagonist (a molecule that binds to the receptor but is unable to activate it) competition. It appears that three types of interactions were observed, depending on the nature of the odorants included and concentration ratios. In the first (and most typical) case, the response intensity of OSNs to the mixture is lower than the response to the most intense component (subtraction or hypo-addition). There seems to be some incongruence in the literature regarding the perception of iso-intense mixtures. Laing *et al.*, (1984) observed that in binary mixtures, both odorants were perceived only when they were similar in intensity. Wilson & Stevenson (2010) observed that mixing two odorants that are perceptually similar may have the effect of doubling the concentration of one odorant, and Atanasova *et al.*, (2005) found that predictive models for odour intensity and quality perception were unable to account for the odour quality dominance in mixtures with iso-intense components. Effects are also difficult to predict if concentrations are dissimilar. Chaput *et al.*, (2012) showed competitive interaction in wine between whiskey lactone and isoamyl acetate, with the perception of 'fruity notes' of a mixture increased by low concentrations of whiskey lactone and decreased by high concentrations. Barkat *et al.,* (2012) noted that odorant combinations of two or three components are more inclined to elicit perceptual interactions, with configural processing conferring an odour quality modification. Specific training and exposure to odours experienced by experts leads their olfactory systems to engage more readily an elemental processing of odour mixtures, and they thus have the ability to better 'select out' individual components from odour mixtures. This does not seem to have been well-tested in the literature.

2.4.2 Olfactory interaction in red wine matrices: previous findings

Pineau & Barbe (2009) demonstrated that in mixtures in de-aromatised red wine, very small variations in the concentrations of some ethyl esters were perceived, even at concentrations far below their individual olfactory thresholds, and affected 'red-berry' and 'blackberry' aromas. Lytra *et al.*, (2012) used omission tests to show that ethyl 2-hydroxy-4-methylpentanoate was responsible for enhancing 'blackberry' and 'fresh-fruit' aromas. These workers also established that a combination of diacetyl, acetoin, acetic acid, and γ-butyrolactone, at levels between 2 and 40% of their perception thresholds had hypo-additive or synergistic effects on the 'fresh fruity' aroma of red wine. Lytra *et al.,* (2013) also showed that ethyl-3-hydroxybutanoate and 2-methylpropylacetate in model wine mixtures led to a significant decrease in the olfactory threshold of the 'fruity' aroma pool, demonstrating synergistic effect in increasing the overall intensity. The authors concluded that compounds with similar chemical structures participated in modulating fruity aromas, specifically the 'berry' and 'fresh-fruit' aromas. Ferreira *et al*, (2002) observed an additive effect between furaneol and homofuraneol in reconstitution studies in red wines. In a larger study (2016) the same author noted that individual compounds in de-aromatised red wine explained only 15% of the sensory effects. Norisoprenoids (β-damascenone and α-ionone) were observed to influence the perception of 'dried-' and 'black fruits' and suppress 'red fruits'. Branched acids (2-methylpropanoic acid and 2- and 3-methyl butyric acid) were shown to suppress 'black fruit' aroma and enhance 'red-' and 'dried fruits'. Strong suppressors of 'red fruit' attributes and 'woody' notes included 2- and 3- methyl propanol. These examples emphasise the importance of perceptual interactions on the intensity and quality of fruity aromas in the wine, but do not characterise any new 'odour-object' formation as a result of perceptual interactions of the compounds.

Recent efforts into volatile phenol (VP) related off-flavours have been concentrated on smoke-related attributes, and a number of studies have an analytical focus. It has been established that VPs at low levels can be detrimental to wine quality (Panzeri, 2013). Descriptors of various VPs spiked into wine were established during the training sessions in this study and included 'tar-like' and 'chemical' for the cresols, to 'sick sweet' and 'medicinal' of the xylenols (Panzeri, 2013). Descriptive analysis was also used to characterise interactions between phenol, *o*-cresol, 3,4-xylenol and 4-EP at low levels. Descriptors included 'smoky-ash', 'medicinal/ Bandaid', 'burnt rubber' and 'sick-sweet'. Interestingly, in this study, phenol itself in red wine was described as 'floral' and 'sweet', enhancing the berry jam character of the Pinotage base wine. Other studies looking at the interactions between VPs or with other taint compounds are scarce. Lorrain *et al.,* (2013) and Tempere *et al.,* (2016) investigated the masking effects of ethylphenols on fruity odours. In one of the few studies conducted in wine on the effect of TCA, Tempere *et al.*, (2017) investigated the masking effect of subthreshold concentrations of TCA on a range of aromatic notes. They showed that TCA caused 'counteraction of odorant specificity', or that low levels made other odours more difficult to identify. Tempere's results suggested that the TCA interaction takes place at receptor level, and that their study provided experimental confirmation of the widespread idea that constituents in non-perceptible concentrations influence the perceived quality of mixtures of odorous compounds.

Even with a compound as well known as IBMP for its anecdotal masking (antagonistic/ 'scalping') effect of IBMP, studies in red wine are surprisingly scarce. Van Wyngaard *et al.*, (2014) investigated the interaction between IBMP and 3-mercaptohexan-1-ol (3MH) in dearomatised Sauvignon Blanc wine, and found that IBMP suppressed the tropical attributes associated with 3MH and that 3MH suppressed the green attributes that correlated with IBMP. The concentrations at which the suppression occurred and the degree of suppression was different for each attribute. Other than Lapalus (2016) study on quantifying and characterising IBMP in Cabernet Sauvignon, studies on its effect and interactions in wine are rare in the primary literature.

3. The choice of methodology in evaluating effects of olfactory interactions

Sometimes chemical analysis of wines labelled as 'tainted' by international experts do not reveal elevated levels of compounds (for example, pyrazines, sulphides, or ethyl acetate) usually associated with fault descriptors. The assumption is often that compounds only pose a problem if they are above their ODT, and malodourous compounds may be present in insufficient quantities (according to chemical analysis) to be perceived as a threat to wine quality. This is the rationale behind the concept of Odour Activity Values (OAVs), a measure that is frequently used by wine researchers, oenologists and flavour chemists (Atanasova *et al.*, 2004; Styger *et al.*, 2011; Ferreira, 2012; Yang *et al.*, 2015). However, it is frequently the case that the OAVs or ODTs do not fully elucidate what is happening in the aroma profile of the wine (Panzeri, 2013; Lapalus, 2016; Wilson, 2017) so sensory evaluation is essential. The choice of sensory methodology is critical to the type of study undertaken, and will determine the nature of the information derived from the evaluation exercise (Meilgaard *et al.*, 2016; Weightman *et al.*, 2016).

When selecting a sensory method, researchers need to take into account the value and detail of information gained versus the costs of gaining such knowledge (Wilson, 2017). Descriptive Analysis (DA) is undoubtedly one of the most sophisticated, flexible and widely used tools in the field of sensory science (Kemp *et al.*, 2018). DA is ideal for evaluating wines as the method is able to provide quantitative information (through the intensity ratings and consensus training) about products that may have only small differences between them (Lawless & Heymann, 2010). As it is widely recognised as a sensory tool, new methodologies will usually be measured against DA in terms of their performance (Torri *et al.*, 2013; Hopfer & Heymann, 2014; Mielby *et al.*, 2014). For all its advantages and sensitivity, DA is time-consuming, expensive and relies on extensive training and diligent attendance on the part of a large panel, and an experienced and highly skilled panel-leader (Kemp *et al.*, 2018). Perrin *et al.*, 2007 noted that in the wine industry the methods classically used in sensory analysis are not always practical as winemakers are not available as sensory panellists for the intensive training on extended studies required by sensory researchers.

Time-effective "rapid methods" have been developed and popularised within sensory research to address these issues. Projective mapping (PM) was originally suggested as a companion method to DA (Pagès, 2005). PM consists of positioning products on a sheet of blank paper simultaneously in such a way that wines are placed near each other if they are perceived as identical and distant from one another if they are perceived as different. Each judge chooses their own sensory criteria and assign their own importance to the criteria. The method provides Euclidian configurations for each attribute for subjects/judges and wines. A number of studies have compared the information gained from PM studies to those from DA and have found rapid methods accurate and reliable (Perrin *et al.*, 2007; Dehlholm *et al.*, 2012; Hopfer & Heymann, 2014). PM is useful to the wine profession because of its speed of execution and flexibility, especially when differences between products do not need to be quantitatively communicated, but it should be noted that data capturing can be time-consuming as judge-responses/product positions need to be converted to coordinates that are then subject to multi-factorial statistical analysis. The number of judges needed in a sensory evaluation exercise has elicited discussion in the literature (Meilgaard, 1993; Lopes *et al.*, 2009; Lawless & Heymann, 2010a; Hopfer & Heymann, 2014; Silva *et al.*, 2014) but for rapid methods, it is generally agreed that 10-11 judges are necessary for this specific set of parameters, as long as three replicates are carried out (Silva *et al.*, 2014). Although rapid sensory methodologies have received attention in the primary and popular wine literature (Risvik *et al.*, 1994; Pagés, 2003; Dalgleish *et al.*, 2007; Holt & Pearson, 2014; Coulon-Leroy *et al.*, 2017; Brand *et al.*, 2018;), there is still a lot of work needed to fully describe the applicability of particular methods to various evaluation scenarios in wine tasting.

4. Conclusions

Perceptive interaction phenomena between aroma compounds in red wines represent an important source of complexity, and emphasise the importance of the matrix and consideration of other compounds in solution when carrying out sensory studies. This area warrants a lot more attention from oenology researchers, particularly in the arena of off-odours and taints, as few studies exist that help to elucidate effects of important contributors.

Despite the importance of wine quality to consumers, and the increasing sensitivity and availability of chemical methods for testing for contributing compounds, there are surprisingly few formal studies quantifying or qualifying off-flavours. A lot of the existing information is anecdotal. The studies that have been carried out are centered on more obvious and easily perceived wine flaws, neglecting faults that do not present a simple, easily recognisable profile. Examples include ‘chemical-related’, ‘vegetal-related’, ‘burnt rubber/ acrid’, and ‘animal-related’ that have still not been addressed. There is also a scarcity of studies in the area of ‘green’ and ‘herbaceous’ off-flavours in wine that cannot be explained by compositional data, especially those that may be related to additive or subtractive effects of interactions. A number of authors have emphasised the need to understand the ‘green’ off-flavours in wine, and carry out studies in this area.

Although the effects of the compounds most frequently associated with smoke-taint in wine (guaiacol and 4-methyl guaiacol) have been well described, effects of certain volatile phenols (VPs) that are known to be produced during smoke events (for example the xylenols) have not yet been well-documented in wine. Little information exists on the majority of the alkylated volatile phenols in wine, despite these compounds having strong odours, and being present in wine as a result of smoke taint or other contamination. Despite comprehensive studies on many aspects of VPs in smoke taint, particularly by Australian researchers, most VPs have not been formally characterised in any wine matrix or assigned threshold values as a result of a formal determination study. Even within a broad category (white or red), the wine-style and choice of cultivar may alter detection thresholds, but evidence from formal studies looking at this aspect is hard to find. Even something as obvious as %v/v alcohol can affect perception of thresholds of odour compounds, but surprisingly few studies exist to examine this aspect in wine. These may be the result of interactions of low levels compounds across different chemical groups, but a significant gap exists in the literature in this regard specifically at levels below detection threshold. Interactions between VPs themselves is an area that requires attention, as well as whether VPs could contribute to other issues like herbaceousness through interactions with other compounds including IBMP and TCA. The effect of various wine matrices on ODTs for VPs, IBMP and TCA, or ODTs of less common VPS that may well have important sensory effects (e. g. phenol and xylenols) in red wine, but there has been little research in this area. Although rapid sensory methodologies have received attention in the primary and popular wine literature, there is still a lot of work needed to fully describe the applicability of particular methods to various evaluation scenarios in wine tasting.

At a more fundamental level, elucidating meaningful links between even simple chemical structures and perceived odours from sensory data, the so-called ‘structure-odour-relationships’ is still not well-documented, even for simple aromatic compounds. Studies on the role of functional groups in the perception of odour, characterisation of single compounds at various levels and the perception of odour-interactions in wine are lacking. Modern computational approaches and access to thousands of physicochemical configurations and odour attributes have provided some knowledge, but a lot of questions remain unanswered. The olfactory perception space is nebulous, nomenclature is arbitrary, and odour classes are overlapping, complex and confusing.

REFERENCES

Albert, P., Kidd, M., *et al.,* 2013. Quantitative Survey of 3-alkyl-2-methoxypyrazines and First Confirmation of 3-ethyl-2-methoxypyrazine in South African Sauvignon blanc Wines. South African J. Enol. Vitic. 34, 1, 54-67.

Allen, M. & Lacey, M., 1996. Methoxypyrazines: New insights into their biosynthesis and occurrence In: New York State Agricultural Experiment Station (ed). Proc. Fourth Int. Symp. Cool Clim. Enol. Vitic. Geneva, N.Y., New York State Agricultural Experiment Station, Rochester Riverside Convention Centre, Rochester, N.Y.

Allen, M., Lacey, M., *et al.,* 1996. Existence of different origins for Methoxypyrazines of grapes and wines. In: G.R. Takeoka, R. Teranishi, P.J. Williams, & A. Kobayashi (eds). Biotechnol. Improv. Foods Flavors. American Chemical Society, Washington DC 220–227.

Alvarez-Rodríguez, M.L., López-Ocaña, L., *et al.,* 2002. Cork taint of wines: role of the filamentous fungi isolated from cork in the formation of 2,4,6-trichloroanisole by O methylation of 2,4,6-trichlorophenol. Appl. Environ. Microbiol. 68, 12, 5860–9.

Atanasova, B., Thomas-Danguin, T., *et al.,* 2004. Perceptual interactions between fruity and woody notes of wine Flavour Frag. J. 19, 6, 476–482.

Atanasova, B., Thomas-Danguin, T., *et al.,* 2005. Perception of wine fruity and woody notes: influence of peri-threshold odorants. Food Qual. Prefer. 16, 6, 504–510.

Audouin, V., Bonnet, F., *et al.,* 2001. Limitations in the Use of odor activity values to determine important odorants in foods. ACS Symposium Series 782: March, 156-171.

Ayabe-Kanamura, S., Schicker, I., *et al.,* 1998. Differences in Perception of Everyday Odors: a Japanese-German Cross-cultural Study. Chem. Sense, 23, 31-38.

Le Barbé, E., 2003. Creating and Validating an aroma and flavor lexicon for the evaluation of sparkling wines. Master's Thesis. University of California, Davis, California, USA.

Barkat, S., Le Berre, E., *et al.,* 2012. Perceptual Blending in odor mixtures depends on the nature of odorants and human olfactory expertise. Chem. Senses 37, 2, 159–166.

Bearak, B., 2009. A Whiff of Controversy and South African Wines - The New York Times Available at https://www.nytimes.com/2009/06/29/world/africa/29stellenbosch.html.

Le Berre, E., Thomas-Danguin, T., *et al.,* 2007. Perceptual processing strategy and exposure influence the perception of odor mixtures. Chem. Senses 33, 2, 193–199.

Boidron, J., Chatonnet, P., *et al.,* 1988. Influence du bois sur certaines substances odorantes des vins Connaiss. la vigne du vin. 22, 4, 275–294.

Botha, J., 2010. Sensory, chemical and consumer analysis of Brettanomyces spoilage in South African wines. Master's Thesis, Stellenbosch University, Western Province, South Africa.

Brand, J. *et al*. Sorting in Combination with quality Scoring: A Tool for Industry Professionals to Identify Drivers of Wine Quality Rapidly. South African J. of Enol. Viti., [S.l.], 39, n. 2, p. 163-175, sep. 2018. doi:https://doi.org/10.21548/39-2-3203.

Brattoli, M., de Gennaro, G., *et al.,* 2011. Odour detection methods: olfactometry and chemical sensors. Sensors (Basel). 11, 5, 5290–322.

Buser, H.R., Zanier, C., *et al.,* 1982. Identification of 2,4,6-trichloroanisole as a potent compound causing cork taint in wine J. Agric. Food Chem. 30, 2, 359–362.

Cadahía, E., Fernández de Simón, B., *et al*., 2003. Volatile Compounds in Spanish, French, and American Oak Woods after Natural Seasoning and Toasting. J. Agric. Food Chem. 51, 20, 5923–5932.

Cain, W.S., de Wijk, R., *et al.,* 1998. Odor Identification: Perceptual and Semantic Dimensions. Chem. Senses 23, 3, 309–326.

Cameleyre, M., Lytra, G., *et al.,* 2018. Perceptive interactions in red wines: How physico-chemical pre-sensorial effects may affect red wine fruity aromatic expression? In: B. Siegmund & E. Letner (eds). Flavour Sci. (1st ed.). Technischen Universitate Graz, Graz, Germany 241–244.

Chaput, M.A., El Mountassir, F., *et al.,* 2012. Interactions of odorants with olfactory receptors and receptor neurons match the perceptual dynamics observed for woody and fruity odorant mixtures Eur. J. Neurosci. 35, 4, 584–597.

Chatonnet, P., Dubourdie, D., *et al*., 1992. The origin of ethylphenols in wines. J. Sci. Food Agric. 60, 2, 165– 178.

Chrea, C., Valentin, D., *et al.,* 2004. Culture and odor categorization: Agreement between cultures depends upon the odors Food Qual. Prefer. 15, 7–8, 669–679.

Christiaens, J.F., Franco, L.M., *et al.,* 2014. The fungal aroma Gene ATF1 promotes dispersal of yeast cells through insect vectors Cell Rep. 9, 2, 425–432.

Coulon-Leroy, C., Symoneaux, R., *et al.,* 2017. Mixed profiling: A new tool of sensory analysis in a professional context. Application to wines. Food Qual. Prefer. 57, 8–16.

Cravero, M., Bonello, F., *et al.,* 2015. The sensory evaluation of 2,4,6-trichloroanisole in wines J. Inst. Brew. 121, 3, 411–417.

Culleré, L., Cacho, J., *et al.,* 2007. An Assessment of the Role Played by Some Oxidation-Related Aldehydes in Wine Aroma J. Agric. Food Chem. 55, 3, 876–881.

Curtin, C., Bramley, B., *et al.,* 2008. Sensory perception of Brett and relationship to consumer preference In: I.. Blair, R.J and Pretorius (ed). Proc. 13th Aust. Wine Ind. Tech. Conf. Australian Wine Industry Techn. conf., Adelaide 207–211.

Czerny, M., Christlbauer, M., *et al*., 2008. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions Eur. Food Res. Technol. 228, 2, 265–273.

Czerny, M., Brueckner, R., *et al*., 2011. The influence of molecular structure on odor qualities and odor detection thresholds of volatile alkylated phenols. Chem. Senses 36, 6, 539–553.

Dalgleish, T., Williams, J.., *et al*., 2007. Rapid Profiling Technique: Napping. J. Exp. Psychol. Gen. 136, 1, 23–42.

Dehlholm, C., Brockhoff, B., *et al*., 2012. Rapid descriptive sensory methods-Comparison of Free Multiple Sorting, Partial Napping, Napping, Flash Profiling and conventional profiling Food Qual. Prefer. 26, 2, 267–277..

De Vries, C.J., Buica, A., et al., 2016. The Impact of Smoke From Vegetation Fires on Sensory Characteristics of Cabernet Sauvignon Wines Made From Affected Grapes South African J. Enol. Vitic. 37, 1, 22–30.

Distel, H., Ayabe-Kanamura, S., *et al*., 1999. Perception of everyday odors - Correlation between intensity, familiarity and strength of Hedonic judgement. Chem. Senses 24, 2, 191–199.

Van Eeden, P., 2009. Chemical, sensory and consumer analysis of cork taint in South African wines. Master’s Thesis, Stellenbosch University, Western Province, South Africa.

Eisele, T.A. & Semon, M.J., 2006. Best Estimated Aroma and Taste Detection Threshold for Guaiacol in Water and Apple Juice. J. Food Sci. 70, 4, S267–S269.

Escudero, A., Campo, E., *et al*., 2007. Analytical Characterization of the Aroma of Five Premium Red Wines. Insights into the Role of Odor Families and the Concept of Fruitiness of Wines. J. Agric. Food Chem. 55 (11), pp 4501–4510.

Etievant, P.X., 1981. Volatile phenol determination in wine. J. Agric. Food Chem. 29, 1, 65–67.

Ferdenzi, C., Poncelet, J., *et al*., 2014. Repeated exposure to odors induces affective habituation of perception and sniffing. Front. Behav. Neurosci. 8, 119.

Fernandez de Simon, B., Cadahia, E., *et al.,* 2008. Volatile Compounds and Sensorial Characterization of Wines from Four Spanish Denominations of Origin, Aged in Spanish Rebollo (Quercus pyrenaica Willd.) Oak Wood Barrels. J. Agric. Food Chem. 56, 9046–9055.

Ferreira, V., 2012a. Revisiting psychophysical work on the quantitative and qualitative odour properties of simple odour mixtures: a flavour chemistry view. Part 2: qualitative aspects. A review. Flavour Frag. J. 27, 3, 201–215.

Ferreira, V., 2012b. Revisiting psychophysical work on the quantitative and qualitative odour properties of simple odour mixtures: a flavour chemistry view. Part 1: intensity and detectability. A review. Flavour Frag. J. 27, 124–140.

Ferreira, V., Ortín, N., *et al*., 2002. Chemical characterization of the aroma of Grenache rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. J. Agric. Food Chem. 50, 14, 4048–54.

Ferreira, V., Sáenz-Navajas, M.-P., *et al.,* 2016. Sensory interactions between six common aroma vectors explain four main red wine aroma nuances. Food Chem. 199, 447–456.

Franco-Luesma, E. & Ferreira, V., 2016. Reductive off-odors in wines: Formation and release of H_2S and methanethiol during the accelerated anoxic storage of wines Food Chem. 199, 42–50.

Fried, J., Torn, M., *et a*l., 2004. The Impact of Climate Change on Wildfire Severity: A Regional Forecast for Northern California. Clim. Change 64, 1/2, 169–191.

Fudge, A.L., Ristic, R., *et al*., 2011. Amelioration of smoke taint in wine by reverse osmosis and solid phase adsorption. Aust. J. Grape Wine Res. 17, 2, 41–48.

Fugelsang, K.C. & Edwards, C.G. (2007). Wine Microbiology. Prac- tical Applications and Procedures. New York, NY, USA: Springer Science+Business Media.

Goldammer, J., Statheropoulos, M., *et al*., 2009. Impacts of Vegetation Fire Emissions on the Environment, Human Health, and Security: A Global Perspective Dev. Env.Sci. 8, 3–36 .

Goldner, M., Zamora, M., *et al*., 2009. Effect of ethanol level on the perception of aroma compounds and the detection of attributes in red wine. J. Sens. Stud. 24, 2, 243–257.

Goode, J., 2008. Burnt Rubber: The great South African wine debate. Available at http://www.wineanorak.com/blog/2008/10/burnt-rubber-great-south-african-wine.html.

Griffiths, N., 1974. Sensory properties of the chloroanisoles. Chem. Senses 1, 2, 187–195.

Haddad, R., Weiss, T., *et al*., 2010. Global features of neural activity in the olfactory system form a parallel code that predicts olfactory behavior and perception. J. Neurosci. 30, 27, 9017–26.

Hammond, C.E., 2015. South African Wine Under Fire Available at http://www.carolynevanshammond.com/blog/2015/10/12/south-african-wine-under-fire-1.

Hayasaka, Y., Baldock, G.A., Parker, M., *et al*., (2010) Glycosylation of smoke-derived volatile phenols in grapes as a consequence of grapevine exposure to bushfire smoke. Journal of Agricultural and Food Chemistry 58, 10989–10998.

He, J. Qin, Q. *et al*., (2013) The effect of wine closures on volatile sulfur and other compounds during post-bottle ageing. Flavour Frag J. (S.I.): 13th Weurman Flavour Research Symposium, Zaragoza, Spain, 27th–30th September 2011 of the, edited by Vicente Ferreira (University of Zaragoza). https://doi.org/10.1002/ffj.3137

Hein, K., Ebeler, S., *et al*., 2009. Perception of fruity and vegetative aromas in red wine. J. Sens. Stud. 24, 3, 441–455.

Heyns, E., 2014. The Green South African palate - When does mint become eucalyptus or even downright weedy? Wineland Magazine. Available at http://www.wineland.co.za/the-green-south-african-palate-when-does-mint-become-eucalyptus-or-even-downright-weedy/.

Holt, H. & Pearson, W., 2014. Napping – a rapid method for sensory analysis of wines. AWRI Tech. Rev 208, 10–14.

Hopfer, H. & Heymann, H., 2014. Judging wine quality: Do we need experts, consumers or trained panelists? Food Qual. Prefer. 32, 221–233.

Howard, J. & Gottfried, J., 2014. Configural and elemental coding of natural odor mixture components in the human brain. Neuron 84, 4, 857–69.

Jackson, R.S. 2014. Sensory Perception and Wine Assessment: Chapter 11. Wine Science, Fourth Edition. Academic Press, Elsevier, United States. 831-888; DOI: http://dx.doi.org/10.1016/B978-0-12-381468-5.00011-7

Kaeppler, K. & Mueller, F., 2013. Odor Classification: A Review of Factors Influencing Perception-Based Odor Arrangements Chem. Senses 38, 3, 189–209.

Keller, A., Zhuang, H., *et al*., 2007. Genetic variation in a human odorant receptor alters odour perception Nature 449, 468.

Keller, A., Gerkin, R., *et al*., 2017. Predicting human olfactory perception from chemical features of odor molecules. Science.355, 6327, 820–826.

Kelly, D. & Zerihun, A., 2015. The effect of phenol composition on the sensory profile of smoke affected wines Molecules 20, 6, 9536–9549.

Kelly, D., Zerihun, A., *et al*., 2012. Exposure of grapes to smoke of vegetation with varying lignin composition and accretion of lignin derived putative smoke taint compounds in wine Food Chem. 135, 2, 787–798.

Kelly, D., Zerihun, A., *et al*., 2014. Winemaking practice affects the extraction of smoke-borne phenols from grapes into wines Aust. J. Grape Wine Res. 20, 3.

Kemp, S.E., Ng, M., *et al.,* 2018. Introduction to Descriptive Analysis In: Descr. Anal. Sens. Eval. John Wiley & Sons, Ltd, Chichester, UK 1–39.

Kennison, K., Wilkinson, K., *et al*., 2008. Smoke-derived Taint in Wine: Effect of Postharvest Smoke Exposure of Grapes on the Chemical Composition and Sensory Characteristics of Wine J. Agric. Food Chem. 55, 26, 10897–10901.

Kennison, K., Wilkinson, K., *et al*., 2009. Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine Aust. J. Grape Wine Res. 15, 228–237.

Kennison, K., Wilkinson, K., *et al*., 2011. Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties Aust. J. Grape Wine Res. 17, 2, S5–S12.

Krstic, M., Johnson, D., *et al*., 2015. Review of smoke taint in wine: Smoke-derived volatile phenols and their glycosidic metabolites in grapes and vines as biomarkers for smoke exposure and their role in the sensory perception of smoke taint Aust. J. Grape Wine Res. 21, 537–553.

Laing, D.G., Panhuber, H., *et al.,* 1984. Quality and intensity of binary odor mixtures Physiol. Behav. 33, 2, 309–319.

Laing, D.G., Eddy, A., *et al.,* 1994. Perceptual characteristics of binary, trinary, and quaternary odor mixtures consisting of unpleasant constituents Physiol. Behav. 56, 1, 81–93.

Lapalus, E., 2016. Linking sensory attributes to selected aroma compounds in South African Cabernet Sauvignon wines, Master’s Thesis, Stellenbosch University, Western Province, South Africa.

Lawless, H., 1999. Descriptive analysis of complex odors: reality, model or illusion? Food Qual. Prefer. 10, 4–5, 325–332.

Lawless, H. & Heymann, H., 2010a. Measurement of Sensory Thresholds. (2nd ed.). Springer Science & Business Media (Food Science Text Series), New York.

Lawless, H. & Heymann, H., 2010b. Sensory Evaluation of Foods: Principles & Practices. (2nd ed.). Springer Science Business Media LLC, New York.

Livermore, A. & Laing, D.G., 1998. The influence of odor type on the discrimination and identification of odorants in multicomponent odor mixtures. Physiol. Behav. 65, 2, 311–20.

Lopes, P., Silva, M., *et al*., 2009. Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon blanc wine during bottle storage J. Agric. Food Chem. 57, 21, 10261–10270.

Lorrain, B., Tempere, S., *et al.,* 2013. Influence of phenolic compounds on the sensorial perception and volatility of red wine esters in model solution: An insight at the molecular level Food Chem. 140, 1–2, 76–82.

Lytra, G., Tempere, S., *et al.,* 2012. Impact of Perceptive Interactions on Red Wine Fruity Aroma J. Agric. Food Chem. 60, 50, 12260–12269.

Lytra, G., Tempere, S., *et al*., 2013. Study of Sensory Interactions among Red Wine Fruity Esters in a Model Solution J. Agric. Food Chem. 61, 36, 8504–8513.

Malfeito-Ferreira, M., Barata, A. & Loureiro, V. (2009). Wine spoilage by fungal metabolites. In: Wine Chemistry and Biochemistry. (Edited by M.V. Moreno-Arribas & M.C. Polo) New York, NY, USA: Springer Science+Business Media

Marais, J. & Swart, E., 1999. Sensory Impact of 2-Methoxy-3-Isobutylpyrazine and 4-Mercapto-4-Methylpentan-2-One Added to a Neutral Sauvignon blanc Wine South African J. Enol. Vitic. 20, 2, 77–79.

de March, C.A., Ryu, S.E., *et al.,* 2015. Structure-odour relationships reviewed in the postgenomic era Flavour Frag. J. 30, 5, 342–361.

Martineau, B., Acree, T., *et al.,* 1995. Effect of wine type on the detection threshold for diacetyl Food Res. Int. 28, 2, 139–143.

Mazzoleni, V. & Maggi, L., 2007. Effect of wine style on the perception of 2,4,6-trichloroanisole, a compound related to cork taint in wine. Food Res. Int. 40, 6, 694–699.

Meilgaard, M.C., 1993. Individual differences in sensory threshold for aroma chemicals added to beer. Food Qual. Prefer. 4, 3, 153–167.

Meilgaard, M.C., Civille, G., *et al.,* 2016. Sensory evaluation techniques. (Fifth ed.). CRC Press, Taylor & Francis Group, New York.

Mielby, L.H., Hopfer, H., *et al.,* 2014. Comparison of descriptive analysis, projective mapping and sorting performed on pictures of fruit and vegetable mixes Food Qual. Prefer. 35, 86–94.

Mozzon, M., Savini, S., *et al.,* 2016. The herbaceous character of wines Ital. J. Food Sci. 28, 2, 190–207. Munch, D., Schmeichel, B., *et al.*, 2013. Weaker Ligands Can Dominate an Odor Blend due to Syntopic Interactions Chem. Senses 38, 4, 293–304.

Noble, A.C., Arnold, R.A., *et al*., 1987. Modification of a Standardized System of Wine Aroma Terminology Am. J. Enol. Vitic 38, 2.

Oelofse, A., Lonvaud-Funel, A., *et al*., 2009. Molecular identification of *Brettanomyces bruxellensis* strains isolated from red wines and volatile phenol production Food Microbiol. 26, 4, 377–385.

Pagès, J., 2005. Collection and analysis of perceived product inter-distances using multiple factor analysis: Application to the study of 10 white wines from the Loire Valley Food Qual. Prefer. 16, 7, 642–649.

Pangborn, R.M., Guinard, J.-X., *et al*., 1988. Regional aroma preferences Food Qual. Prefer. 1, 1, 11–19.

Panzeri, V., 2013. Influence of vineyard posts type on the chemical and sensorial composition of Sauvignon blanc and Merlot Noir wines. Master's Thesis, Stellenbosch University, Western Cape, South Africa

Parker, B., Baldock, G., *et al*., 2013. Seeing through smoke. Wine Vitic. J. January/Feb, 28, 42–46.

Parker, M., Osidacz, P., *et al*., 2012. Contribution of Several Volatile Phenols and Their Glycoconjugates to Smoke-Related Sensory Properties of Red Wine J. Agric. Food Chem 60, 2629–2637.

Parr, W., Mouret, M., *et al*., 2011. Representation of complexity in wine: Influence of expertise Food Qual.Prefer. 22, 7, 647–660.

Parr, W. V., Green, J.A., *et al*., 2007. The distinctive flavour of New Zealand Sauvignon blanc: Sensory characterisation by wine professionals Food Qual. Prefer. 18, 6, 849–861.

Perrin, L., Symoneaux, R., *et al.,* 2007. Comparison of three sensory methods for use with the Napping procedure: Case of ten wines from Loire valley. Food Qual. Prefer. 19, 1–11.

Perry, D. & Hayes, J., 2016. Effects of matrix composition on detection threshold estimates for Methyl Anthranilate and 2-Aminoacetophenone Foods 5, 2, 35–45.

Petrozziello, M., Asproudi, A., *et al.,* 2014. Influence of the matrix composition on the volatility and sensory perception of 4-ethylphenol and 4-ethylguaiacol in model wine solutions. Food Chem. 149, 197–202.

Pineau, B. & Barbe, J., 2009. Examples of Perceptive Interactions Involved in Specific " Red- " and " Black-berry " Aromas in Red Wines J. Agric. Food Chem. 57, 9, 3702–3708.

Pollnitz, A., Pardon, K., *et al*., 1996. The analysis of 2,4,6-trichloroanisole and other chloroanisoles in tainted wines and corks Aust. J. Grape Wine Res. 2, 3, 184–190.

Pollnitz, A., Pardon, K.H, *et al*., 2004. The Effects of Sample Preparation and Gas Chromatograph Injection Techniques on the Accuracy of Measuring Guaiacol, 4-Methylguaiacol and Other Volatile Oak Compounds in Oak Extracts by Stable Isotope Dilution Analyses. J. Agric. Food Chem., 2004, 52 (11), pp 3244–3252

Prescott, J., Norris, L., *et al*., 2005. Estimating a "consumer rejection threshold" for cork taint in white wine Food Qual. Prefer. 16, 4, 345–349.

Prida, A. & Chatonnet, P., 2010. Impact of oak-derived compounds on olfactory perception of barrel-aged wines Am. J. Enol. Vitic. 50, 4, 447–455.

Rauhut, D. & Kiene, F. (2019). Chapter 19: Aromatic Compounds in Red Varieties. Red Wine Technology, pp273-282. Doi:10.1016/b978-0-12-814399.00019-0

Ristic, R., van der Hulst, L., *et al*., 2017. Impact of Bottle Aging on Smoke-Tainted Wines from Different Grape Cultivars J. Agric. Food Chem. 65, 20, 4146–4152.

Risvik, E., Mcewan, J., *et al.,* 1994. Projective Mapping: A tool for Sensory Analysis and Research Food Qual. Prefer. 5, 263–269.

Romano, A., Perello, M.C., *et al*., 2009. Sensory and analytical re-evaluation of “Brett character” Food Chem.114, 1, 15–19.

Roujou De Boubée, D., Van Leeuwen, C., *et al*., 2000. Organoleptic Impact of 2-Methoxy3-isobutylpyrazine on Red Bordeaux and Loire Wines. Effect of Environmental Conditions on Concentrations in Grapes during Ripening J. Agric. Food Chem. 48, 4830–4834.

Sefton, M. & Simpson, R., 2005. Compounds causing cork taint and the factors affecting their transfer from natural cork closures to wine – a review Aust. J. Grape Wine Res. 11, 2, 226–240.

Sell, C., 2014. The Mechanism of Olfaction In: Wiley (ed). Chem. Sense Smell. (1st ed.). John Wiley & Sons, Inc., Hoboken, NJ, USA 32–187.

Sell, C.S., 2006. On the Unpredictability of Odor Angew. Chemie Int. Ed. 45, 38, 6254–6261. Shibamoto, T., 1986. Odor Threshold of Some Pyrazines J. Food Sci. 51, 4, 1098–1099.

Shutz, M. & Kunkee, R., 1977. Formation of Hydrogen Sulfide from elemental sulfur during winemaking Am.J. Enol. Vitic. 28, 3, 137–144.

Silva, R. de C. dos S.N. d, Minim, V.P.R., *et al*., 2014. Number of judges necessary for descriptive sensory tests Food Qual. Prefer. 31, 22–27.

Silva Teixeira, C., Cerqueira, N., *et al*., 2015. Unravelling the olfactory sense: From the Gene to Odor Perception. Chem. Senses 41, 2. DOI: 10.1093/chemse/bjv075

Simpson, R., Amon, J., *et al*., 1986. Off-flavour caused by Guaiacol Food Technol. Aust. 38, 31–33.

Spillman, P.J., Iland, P.G., *et al*., 1998. Accumulation of volatile oak compounds in a model wine stored in American and Limousin oak barrels. Aust. J. Grape Wine Res. 4, 2, 67–73.

Strydom, S. & Savage, M., 2016. A spatio-temporal analysis of fires in South Africa South African J. Sci. J. Sci 112, 11, 1–8.

Styger, G., Prior, B., *et al*., 2011. Wine flavor and aroma J. Ind. Microbiol. Biotechnol. 38, 9, 1145–1159.

Suklje, K., K. Lisjak, H.B. Cesnik, L. Jane, W.J. Du Toit, Z. Coetzee, A. Vanzo & A.J. Deloire. 2012. Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazines, glutathione and hydroxycinnamates evolution during berry ripening of Sauvignon blanc (Vitis vinifera L.) Journal of Agricultural and Food Chemistry 60, 9454-9461

Takeuchi, H., Kato, H., *et al*., 2013. 2,4,6-trichloroanisole is a potent suppressor of olfactory signal transduction. Proc. Natl. Acad. Sci. U. S. A. 110, 40, 16235–40.

Taylor, M., Young, T., *et al*., 2000. Supercritical fluid extraction of 2,4,6-trichloroanisole from cork stoppers J. Agric. Food Chem. 48, 6, 2208–2211.

Tempere, S., Cuzange, E., *et al*., 2012. Explicit Sensory Training Improves the Olfactory Sensitivity of Wine Experts Chemosens. Percept. 5, 2, 205–213.

Tempere, S., Cuzange, E., *et al*., 2014. “Brett character” in wine: Is there a consensus among professional assessors? A perceptual and conceptual approach Food Qual. Prefer. 34, 29–36.

Tempere, S., Schaaper, M.H., *et al*., 2016. The olfactory masking effect of ethylphenols: Characterization and elucidation of its origin Food Qual. Prefer. 50, 135–144.

Tempere, S., Hamtat, M., *et al*., 2016. Comparison of the ability of wine experts and novices to identify odorant signals: a new insight in wine expertise Aust. J. Grape Wine Res. 22, 2, 190–196.

Tempere, S., Schaaper, M.H., *et al*., 2017. Masking of Several Olfactory Notes by Infra-threshold Concentrations of 2,4,6-Trichloroanisole Chemosens. Percept. 10, 3, 69–80.

Thomas-Danguin, T., Sinding, C., *et al*., 2014. The perception of odor objects in everyday life: a review on the processing of odor mixtures Front. Psychol. 5, June, 1–18.

Torri, L., Dinnella, C., *et al*., 2013. Projective Mapping for interpreting wine aroma differences as perceived by naïve and experienced assessors Food Qual. Prefer. 29, 1, 6–15.

Vilanova, M. & Oliveira, J., 2012. Application of Gas Chromatography on the Evaluation of Grape and Wine Aroma in Atlantic Viticulture (NW Iberian Peninsula) In: S. Bekir & O. Celikbicak (eds). Gas Chromatogr. Plant Sci. Wine Technol. Toxicol. Some Specif. Appl. InTechOpen, London, United Kingdom.

Weightman, C., Brand, J., *et al*., 2016. Sensory evaluation of wine (Part 1) Available at https://www.wineland.co.za/sensory-evaluation-of-wine-part-1/.

Weiss, S., 2014. The influence of grape variety on the production of volatile phenols in Portuguese wines Universidade Catolica Portuguesa: Escola Superior de Biotecnologia.

Wilkinson, K.L., Ristic, R., *et al*., 2011. Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine Aust. J. Grape Wine Res. 17, S22–S28.

Wilson, C., 2017. Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines Master's Thesis, Stellenbosch University, Western Cape, South Africa.

Wilson, C., Brand, J., *et al*., 2018. Interaction Effects of 3-Mercaptohexan-1-ol (3MH), Linalool and Ethyl Hexanoate on the Aromatic Profile of South African Dry Chenin blanc Wine by Descriptive Analysis (DA) South African J. Enol. Vitic. 39, 2, 271–283.

Wilson, D. & Stevenson, R., 2010. Learning to Smell. (2nd ed.). John Hopkins University Press.

Wilson, D.A., 2005. Odor Perception is Dynamic: Consequences for Interpretation of Odor Maps Chem.Senses 30, Supplement 1, i105–i106.

Wilson, D.A. & Sullivan, R.M., 2011. Cortical Processing of Odor Objects Neuron 72, 4, 506–519.

Wirth, I., Guo, W., *et al*. (2001) Volatile compounds released by enzymatic hydrolysis of glycoconjugates of leaves and grape berries from Vitis vinifera Muscat of Alexandria and Shiraz cultivars. Journal of Agricultural and Food Chemistry 49, 2917–2923.

Wolf, L.., 2018. Wildfires and wine: A detective story Chem. Eng. News 96, 19, 22–25.

Wooding, S., 2013. Olfaction: It Makes a World of Scents Curr. Biol. 23, 16, R677–R679.

Wurz, D.A., Allebrandt, R., *et al*., 2017. Women have better olfactory perception for wine aromas BIO Web Conf. 9, 04005.

van Wyngaard, E., Brand, J., *et al*., 2014. Sensory interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatised Sauvignon Blanc wine Aust. J. Grape Wine Res. 20, 2, 178–185.

Yang, W., Li, W., *et al*., 2015. Odour prediction model using odour activity value from pharmaceutical industry Austrian Contrib. to Vet. Epidemiol. 8, 51–60.

Young, W.F., Horth, H., *et al*., 1996. Taste and odour threshold concentrations of potential potable water contaminants Water Res. 30, 2, 331–340.

Zoecklein, B.W., Fugelsang, K.C., *et al*., 1995. Volatile Acidity In: Wine Anal. Prod. Springer US, Boston, MA 192–198.

Chapter 3

Photo: C. De Vries

Elucidating Chemical and Sensory Effects of Volatile Phenols in Smoke-Affected Red Wines

This article has been submitted to the South African Journal of Enology and Viticulture

Elucidating Chemical and Sensory Effects of Volatile Phenols in Smoke-Affected Red Wines

Marianne McKay [1*], Florian F. Bauer [2], Valeria Panzeri [3,] Lucky Mokwena[4] and Astrid Buica[5]

[1] Department of Viticulture and Oenology; Stellenbosch University, marianne@sun.ac.za

[2] Department of Viticulture and Oenology; Stellenbosch University, fb2@sun.ac.za

[3] Institute for Grape and Wine Sciences; Stellenbosch University panzeri@sun.ac.za

[4] Central Analytical Facility, Stellenbosch University, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa

[5] Department of Viticulture and Oenology; Stellenbosch University abuica@sun.ac.za

***Corresponding author**: marianne@sun.ac.za; Tel.: +27-21-8083774

Submitted for publication:

Accepted for publication:

Key words: volatile phenols; smoke taint; wine sensory evaluation; GC-MS; DA;

Running title: *Effects of Volatile Phenols in Smoke-tainted Red Wines*

Abstract

Malodourous compounds, including volatile phenols (VPs) are frequently found at concentrations below their odour thresholds in wine, and may therefore be considered to present no threat to wine quality. Most investigations into smoke taint quantify compounds by chemical/analytical means, or investigate sensory effects of supra- and peri-threshold contamination in model wine. In this project, twelve wines submitted by the South African industry as 'faulty' and/or smoke tainted were screened for VPs using GC-MS, and characterized using Descriptive Analysis (DA) by a sensory panel highly trained in smoke taint evaluation. Results were compared statistically to elucidate relationships between chemical and sensory characteristics. It was demonstrated, using the combined dataset, that concentration and

composition of VPs in the wines correlated well with certain sensory attributes. Guaiacol was present in most samples at peri- or supra-threshold levels, but did not cause taint unless in combination with other phenols, when it was associated with ‘smoky’, ‘ashy’ and ‘herbaceous’ attributes. Wines with very low levels of VPs showed more sweet-associated aroma characteristics. Wines with supra-threshold levels of VPs showed negative attributes (‘chemical/plastic’, ‘tar/BR’ and ‘medicinal/Elastoplast™’). In some cases, sensory effects (‘earthy/dusty/potato skin’, ‘mouldy/musty’ and ‘cooked veg’) could not be attributed to peri- or supra-threshold concentrations, but seemed to be due to combinations of volatile phenols at subthreshold levels. Associations between negative attributes and historical bushfire events prior to harvest were found for a number of the wines. This study emphasizes the importance of understanding effects of VPs on wine aroma, and escalating awareness and sensitivity to these issues in the wine industry (257 words).

1. Introduction

In order to establish and maintain strong, positive international brands in a fiercely competitive market, it is important that wine producers understand the character of their products and ensure consistency of desirable organoleptic features. Negative attributes in red wine, for example, smoke taint, ‘ashiness’, ‘greenness’/‘herbaceousness’ and ‘burnt rubber (BR)’ have been discussed by various authors (Goode 2008, Hammond 2015, Heyns 2014) and necessitate the investigation of compounds associated with these descriptors.

Volatile phenols (VPs) are a group of compounds that have been associated with smoky, burnt and acrid attributes (Parker, *et al*., 2013). Their presence in wine may derive from a number of sources including grapes, yeast, in particular *Brettanomyces* species (Romano *et al*., 2009; Weiss, 2014), wood maturation (Boidron *et al*., 1988; Prida & Chatonnet, 2010) The cresols, as well as 3, 4-dimethylphenol (3,4-DMP), guaiacol and 4-EP have been linked to lignin pyrolysis during the toasting of oak barrels (Etievant, 1981; Cadahía *et al*., 2003; Fernandez de Simon *et al*., 2008). Although VPs, as noted, may derive from a number of sources, a lot of research in recent years concerning VPs has been centered on smoke taint (Krstic et al., 2015), which is the off-odour that results from exposure of grapes to bushfire smoke.

With bushfires in very close proximity to vineyards in most wine growing areas globally, with recent examples in the United States (Jin *et al*., 2015), Australia (Cox, 2018), the Iberian Peninsula (Barnes, 2018), and South Africa, the contribution of VPs to the pool of taint compounds in grapes and wine has severely escalated (Kennison 2013, Wilkinson *et al,*. 2011). These compounds have been individually characterized in different media by a number of authors, and their Odour Detection Thresholds (ODTs) established in specific matrices (Table 1).

Table 1. Odour Detection Thresholds (ODTs) and aroma descriptors for a range of volatile phenol in different matrices

Volatile Phenol	ODT (µg/L)	Descriptors	Reference
guaiacol	23 [a]	burnt, smoky, toasty, phenolic	Parker *et al*. (2012)
2,6- DMP	400 [c]	sweet, tarry	Verschueren, (1983)
4- MG	21 [a]	sweet-spicy, phenolic, leathery	Czerny, *et al*. (2008)
o-cresol	62 [c]	burnt, smoky, medicinal, tar	Parker *et al*. (2013)
phenol	5900 [a]	sweet, cloying, chemical	Amoore, *et al*. (1976)
4-EG	50 [a]	clove, medicinal, woody, sweet	Petroziello, *et al*. (2014)
m-cresol	68 [b]	leather, rubber, ink	Parker *et al*. (2013)
p-cresol	10 [b]	horse, stable, fecal	Parker *et al*. (2013)
2,3- DMP	500 [a]	ink, sweet, leather	Pubchem (2018)
eugenol	700 [c]	clove, phenolic, sweet	Margalit, (2013)
4-EP	605 [c]	leather, bacon, medicinal, horse	Chatonnet *et al*.(1992)
3,4-DMP	1200 [a]	horse, stable, fecal, ink	Boidron *et al*. (1988)

a. ODT in red wine. b. 'ODT in ethanol solution or model wine (10-12% v/v), c. ODT in water

However, aroma compounds in wine are perceived together, and combinatorial effects have an olfactory impact even when these compounds are present at peri-threshold or sub-threshold levels (Lorrain *et al.*, 2013a). Recent research has shown that beneficial aroma compounds such as thiols produce aromatic changes in wine when they are present in combination (Coetzee *et al.,* 2015, Lapalus *et al.,* 2016), which suggests that malodourous compounds in combination at peri- and sub-threshold levels in wine may also produce variable aromatic effects. Assessment based only on chemical analysis may take only ODT or Odour Activity Values (OAV: concentration of compound in solution divided by its ODT) into account, and overlook the contribution or interactive effects of sub-threshold level compounds. Another aspect that may be overlooked in many studies is the impact of the matrix. In wine, alcohol concentration for example has been shown to affect the volatility of aroma compounds (Petrozziello *et al.*, 2014). This situation is further complicated as contributors at peri- and subthreshold levels may not present an easily recognizable fault profile, and matrix effects also play an important role in how compounds are perceived.

Previous researchers (Kennison *et al.*, 2009; Ristic *et al.*, 2017) have elucidated the presence and characteristics of individual volatile phenols (VPs) in deliberately smoke-tainted (experimental) wine. Some authors have also explored the idea of interactions (Lapalus *et al.*, 2016; Wilson, 2017) or characterized the effects of individual smoke taint compounds in specific matrices (Parker *et al.*, 2013).

In order to address industry needs for VP analysis, and build a body of knowledge regarding smoke taint issues, producers are encouraged to wine submit commercial finished wines and tank samples to the Department of Viticulture and Oenology (DVO), Stellenbosch University (SU) each year following bushfires in regions adjacent to the vineyards. Wines may also have been rejected by consumers or from competitions because of suspected taint issues. To our knowledge, the impact of VPs has not previously been analyzed and characterized both sensorially and chemically in inadvertently smoke-affected commercial wines. The aims of this project were thus to investigate whether the sensory attributes of commercial (actually or potentially smoke-affected) wines as evaluated by a trained panel, could be correlated with VP chemistry, as quantified by gas chromatography-mass spectrometry (GC-MS). Results in this study are presented in terms of sensory and chemical data, and an evaluation of relationships that might exist between them, as well as discussion of whether the results mirrored possible smoke-exposure of grapes. This study may therefore provide useful information to the wine industry through increasing understanding of ways in which problematic compounds (in this case VPs) contribute to sensory characteristics, and elucidating whether sensory predictions can be made from chemical data.

2. Materials and Methods

2.1 Wines

Wine samples (750 mL) were selected for this study from wines submitted during 2016 and 2017 by South African wine producers for sensory evaluation at the DVO and VP analysis at the Central Analytical Facility (CAF) at SU. Producers had indicated that the submitted wines may have had smoke taint issues through vineyard expose to smoke, or as a result of their own informal assessment. Wines were not prescreened before the study (except by producers), and therefore so it was not known if the wines were actually contaminated with VPs. Only red wines were submitted by industry, so no white wines were available for the study.

The selection criteria for wines were as follows: a full 750mL bottle of each wine was available to allow descriptive analysis (DA) by the sensory panel, bottle seals were intact, and relevant details were provided by the producers. Twelve wines were randomly selected from those fulfilling the inclusion criteria, as this was the maximum that could be assessed by a sensory panel in one session, using DA, without incurring sensory fatigue (Campo *et al.*, 2010). These wines, from different South African Wine of Origin (WO) regions (see Table 2, also map Figure 1), were labelled A to L for the purposes of the study.

Table 2. Cultivar, vintage, alcohol concentration and region of origin of commercial South African wine samples selected for chemical and sensory analysis

Wine	**Cultivar**	**Vintage**	**Alcohol % v/v**	**WO Region**
A	Grenache	2015	13.5	Franschhoek
B	Grenache	2016	13.0	Franschhoek
C	Cabernet Sauvignon	2012	14.1	Stellenbosch
D	Cabernet Sauvignon	2014	14.0	De Doorns
E	Cabernet Sauvignon	2016	14.2	Franschhoek
F	Cabernet franc	2016	13.0	Elgin
G	Syrah	2016	13.8	Elgin
H	Merlot	2015	13.5	Helderberg
I	Cabernet Sauvignon	2015	14.0	Durbanville
J	CS –Merlot Blend	2015	14.2	Stellenbosch
K	Merlot-CS Blend	2016	13.0	W. Cape
L	Pinotage	2015	13.7	Durbanville

Alcohol concentrations, provided by the producers, ranged from 13 % v/v to 14.2 % v/v. Before analysis, the wines were kept in the Stellenbosch University 'vinothéque', a bespoke wine storage area with controlled temperature and humidity. As only 750 mL of each wine was available, 50 mL samples for GC-MS were taken during the initial sensory training session, and the remaining wines (700ml) were decanted to smaller bottles to reduce ullage to a minimum, and great care was taken to reduce oxygen exposure. Wines were stored under nitrogen for approximately two weeks in a cold room (15˚C) until sensory testing could take place.

Figure 1. Map of the Western Cape, South Africa, showing approximate origin of South African wines suspected of being smoke tainted and submitted for analysis. Wine codes A-L in red

2.2 Sensory Evaluation

DA was conducted on the twelve wines selected for study. Ten participants, all healthy non-smoking females with an age range of 20-60 years, took part in the sensory evaluation panel sessions. These panelists, who regularly attended sensory evaluation sessions in the DVO, and formed part of a formal 'smoke-taint' panel, had previous experience in DA. Sensory data was obtained for this study in compliance with institutional procedures for sensory evaluation (Ethical Clearance VIT-2018-6570). All participants provided their informed consent before participating in the study.

2.2.1 Sensory training

A combination of consensus and ballot training was conducted before testing two training sessions over a period of one week, with an interval of one days between sessions. As smoke taint work had been carried out with this panel previously, panelists were familiar with a number of smoke-related attributes, and therefore did not require as much training to familiarise themselves with attributes. Reference standards were presented in 50 mL amber glass bottles (Consol glass, RSA) following formulations in Table 3.

Table 3. List of terms used for descriptive analysis and associated preparation method for aroma reference standards used during panel training.

Descriptive term	Formulation or concentration
Berries	Mix of mashed berries (defrosted raspberries, blueberries and blackberries) from Hillcrest Berries farm
Prunes/ raisins	1 prune and several raisins (Safari brand) finely chopped
Floral /Violet	Fruit Sirop De Alpes (Violet), France. 5 mL with 5 mL water.
Herbaceous/green	2 cm^3 of fresh green pepper + 10 mL distilled water with 1ml crushed cut grass added
Leather/barnyard/ animal	Le Nez du Vin® standard 'Horse'. 1 drop on cotton wool
Tobacco	Contents of 1 unsmoked cigarette (Camel)
Vanilla/caramel	5ml of vanilla essence (Robertsons) +1/2 toffee (Toffoluxe)chopped and mixed
Medicinal/ Band-aid® /Elastoplast™	Piece of (Elastoplast™) sticking plaster peeled and cut up into little pieces
Smoky	2 mL of chopped, burnt cork
Cooked vegetable	5 mL canned green bean brine (Koo) + 5 mL canned asparagus brine
Pencil shavings	Around a cm of fresh pencil shavings (Staedtler tradition®)
Earthy/dusty/ potato skin	Used paper potato bag with soil remnants
Ashtray	Smoked Benson & Hedges cigarette butts and ash
Tar/BR	Small dab of creosote (Powafix) in a petri dish, sealed
Mouldy/musty	Le Nez du Vin® standard 'mouldy/earth'. 1 drop on cotton wool.
Soy sauce	5 mL of (Hasty Tasty) soya sauce

For the first thirty minutes of each training session, panelists were asked to re-familiarize themselves with specific aromas. After a break of twenty minutes, panelists were presented with 20 mL of each commercial wine sample in black ISO glasses, and asked to assess wine aroma silently for around 30 minutes, using the attribute lists, but also including any new aromas they perceived that were not on the list. Following this, the panel discussed the attributes of each sample, and differences and similarities between samples, which were noted by the panel leader. These discussions generated a comprehensive list of aroma descriptors that included familiar attributes, but also new attributes that were unique to the wines under study. To conclude the session, there was a detailed discussion of descriptive terms. The panel were also asked to rate the intensity of the various aromatic attributes, and the panel leader noted frequencies and intensities on a whiteboard as the discussion took place. The panel agreed by consensus to include or exclude various odour attributes, and reduce redundant terms, until a simplified list of descriptors was decided upon that described all the odour families present in the wines. The data regarding the descriptors and intensities were collected, sorted and tabulated at the end of each session by the panel leader. A final list of seventeen attributes for testing was confirmed after the last training session.

These attributes, agreed upon through consensus by the panel, included ‘sweet-associated’/ generally positive attributes: ‘berries’, ‘floral/ violets’, ‘prunes/raisins’, ‘vanilla/caramel’, ‘tobacco’, and ‘pencil shavings’. Attributes generally regarded as negative to red wine character were also identified: ‘herbaceous/green’; ‘cooked veg.’, ‘leather/barnyard’, ‘earthy/dusty/potato skin’, ‘smoky’, ‘ashtray’, ‘medicinal/Elastoplast™’ (also called ‘Band-Aid’® by the panel), ‘mouldy/musty’, ‘tar/ burnt rubber (BR)’, and ‘soy sauce’.

2.2.2 Sensory testing

The sensory testing of the twelve wines was carried out in a well-ventilated, well-lit sensory laboratory with a constant temperature of 20°C. Each taster worked in a white isolated booth, and no communication was permitted between tasters. Wine samples of exactly 20 mL were presented to tasters in black ISO 3591:1977 standard tasting glasses (Consol glass, Stellenbosch, South Africa). Glasses were covered with clear inert polystyrene lids (Petri dish, Labsupply, Cape Town, South Africa) to allow equilibration of volatiles in the headspace. The twelve wines were evaluated (for aroma attributes only, in triplicate) over two sessions. Samples were marked with random three digit codes and presented to tasters according to William Latin Square design in a unique, counterbalanced manner to avoid order effects, such as those caused by fatigue or desensitization of panel members. For these reasons, tasters were also asked to pause for fifteen minutes between flights. Tasters assessed the twelve wines according to the prescribed attributes list, and assigned an intensity to the attributes perceived in the wine. Intensities were assigned for each attribute by marking on an unstructured line scale, with 0 as not perceived/lowest rating, and 100 as highest intensity. If an attribute was not present/perceived, the panelist was asked to assign zero on the line scale.

2.3 GC-MS analysis

Wines were analyzed by GC-MS according to a modified version of a previously described method (De Vries et al. 2016b). Twelve VPs were quantified: guaiacol, 2,6-dimethyl phenol (2,6-DMP), 4-methylguaiacol (4-MG), *o*-cresol, phenol, 4-ethylguaiacol (4-EG), *m*-cresol, *p*-cresol, 2,3-dimethylphenol (2,3-DMP), eugenol, 4-ethylphenol (4-EP) and 3,4-dimethylphenol (3,4-DMP).

Stock solutions of 1 mg/L of pure compounds (all reference standards supplied by Sigma-Aldrich/Merck, KGaA, Darmstadt, Germany), were diluted for calibration purposes, creating an 8-point calibration series from 0.05 to 100 µg/L. Three 10 mL aliquots of each wine were transferred into 20 mL SPME glass vials (Gerstel, Mülheim, Germany). An internal standard, deuterated anisole-d8 (methoxybenzene-d8; Sigma-Aldrich/Merck, Darmstadt, Germany), was added to each vial at a concentration of 10 µg/L. Two milliliters of 30% w/v NaCl (Merck, Germany) in ultra-pure distilled water (Millipore, Bedford, MA, USA) was also added to each vial. The vials were sealed with PTFE-lined magnetic crimp caps (Gerstel, Mülheim, Germany), and vortexed (Vortex-Genie® 2; Scientific Industries Inc., NY, USA) for 30 seconds before being placed on the auto-sampler (Thermo Scientific TriPlus RSH). Vials were incubated in the auto-sampler for 5 minutes at 50°C, after which a pink 65µm Polydimethylsiloxane/ Divinylbenzene/ (PDMS/DVB/) 'Stableflex' SPME fiber (Supelco, Belafonte, PA, USA) was exposed to the headspace for 15 minutes at the same temperature. After exposure, the fiber was injected and left for ten minutes in order to allow desorption of volatiles. The injector was operated in splitless mode. Analysis of VPs was performed using a Thermo Scientific trace 1300 gas chromatograph (Anatech, coupled to a Thermo Scientific TSQ 8000 Triple Quadrupole Mass (Anatech Instruments (Pty) Ltd, RSA. The MS-detector was set for acquisition in single reaction monitoring (SRM) mode. Chromatographic separation of the VPs was performed on a polar Zebron ZB-FFAP (30 m, 0.25 mm ID, 0.25 µm film thickness, part number 7HG-G009-11) capillary column. The initial oven temperature was 50 °C, held for 3 minutes, then increased to a final temperature of 250 °C at a rate of 15 °C/min and a final hold time of 3 minutes. The injector, ionization source and transfer line temperatures were maintained at 250°C. Helium at 1 mL/min flow rate was used carrier gas. The emission current of 50 µA was used with argon collision. Compounds were identified by cross-referencing retentions times and mass spectra with the NIST11 spectral library. The limit of detection (LOD) and limit of quantitation (LOD) for analytes were calculated using the slope of the calibration curve for each compound and the the standard deviation of the response at low concentrations (σ) where LOD = 3.3 σ/Slope and LOQ = 10 σ/Slope.

2.4 Data Analysis

A mixed model two-way analysis of variance (ANOVA) was applied to assess the significance of the attributes, descriptive data per compound, and panelists' performance, using both PanelCheck® version 1.2.1 (Nofima, Ås, Norway) and Statistica version 12 (StatSoft Inc., Tulsa, USA). Consensus amongst panelists was assessed by Tucker plots. *Post hoc* Fisher's least significant difference (LSD) and least squares means (LSM) were used to test for significance of sensorial differences between the wines. A P-value threshold of 0.05 ($P < 0.05$) was used to determine statistical significance. Principal component analysis (PCA) biplots and 'heatmaps' were created using mean intensity scores of attributes to show similarities or dissimilarities between wines. To illustrate associations between sensory attributes and VP chemistry, Multiple Factor Analysis (MFA) was performed. Wine sensory data, as well as sensory and chemical interactions were analyzed using Statistica 12 (Dell Software, Texas, USA). ‘Heatmaps’ were generated for sensory and chemical data using R 3.4.2 (R Core Team, 2015).

3. Results

3.1 Sensory results

The twelve wines were evaluated for attributes using DA with a trained sensory panel. Separate ANOVAs were generated for each attribute using a mixed model with panelists as the random effect. Certain attributes were weakly perceived by the panelists in most of the wines and gave very few significant differences ($p>0.05$), and therefore have not been shown. The two most intense fruity/ sweet attributes, ie. ‘berries’ and ‘prunes/ raisins’ can be seen in the LS means diagrams in Figure 2. Wine D was perceived as significantly lower than all other samples in these two attributes. Positive (sweet/ fruity) attributes with low intensities (<20) included ‘vanilla/caramel’, ‘tobacco’, ‘pencil shavings’ and ‘floral/violet’.

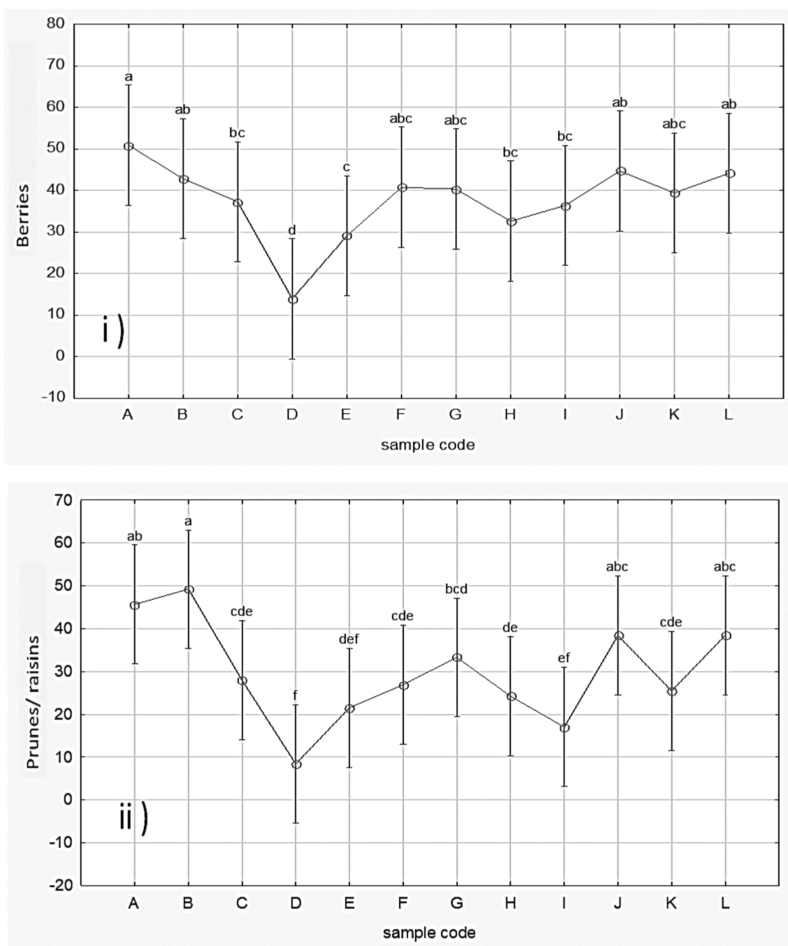


Figure 1. Intensity scores for most intense sweet-associated aroma attributes. i) berries ($p<0.001$) ii) prunes/raisins ($p<0.001$) presented in South African wines (A–L) suspected of being smoke tainted. Values are LS mean scores from 10 judges. Vertical bars denote 0.95 confidence interval.

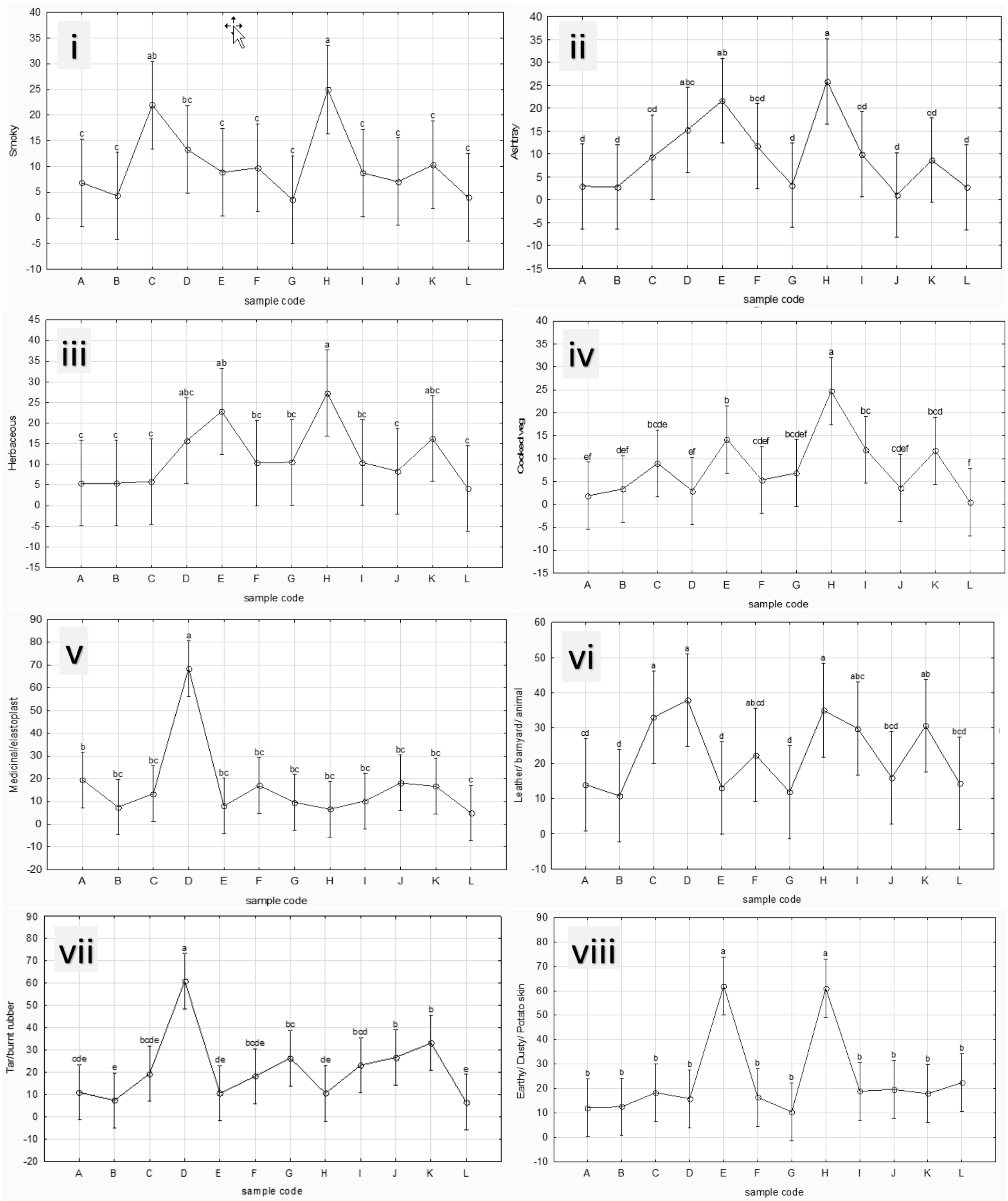


Figure 2. Intensity scores for aroma attributes. i) smoky ii) ashtray iii) herbaceous iv) cooked veg; v) medicinal/Elastoplast™; vi) leather/barnyard/animal; vii) tar/burnt rubber; viii) earthy/dusty/potato skin presented in wines (A–L). Values are mean scores from 10 judges for samples evaluated in triplicate (n=30); different letters indicate statistical significant (LSD post-hoc test), with 95% confidence denoted by vertical bars.

Figure 3 shows selected negative attributes, with wines C, D, E and H presenting these most strongly.

A clustered 'heatmap', a compact means of visualizing large data sets with a number of variables (Perez-Llamas & Lopez-Bigas, 2011), was produced from the sensory data for the twelve wines, giving an holistic picture of their attributes, and providing information on the differences and similarities between the wines (Figure 4). As the sensory data was unitless (0-100 line scale scores for intensity of each attribute), it was not necessary to standardize the dataset before compiling the heatmap.

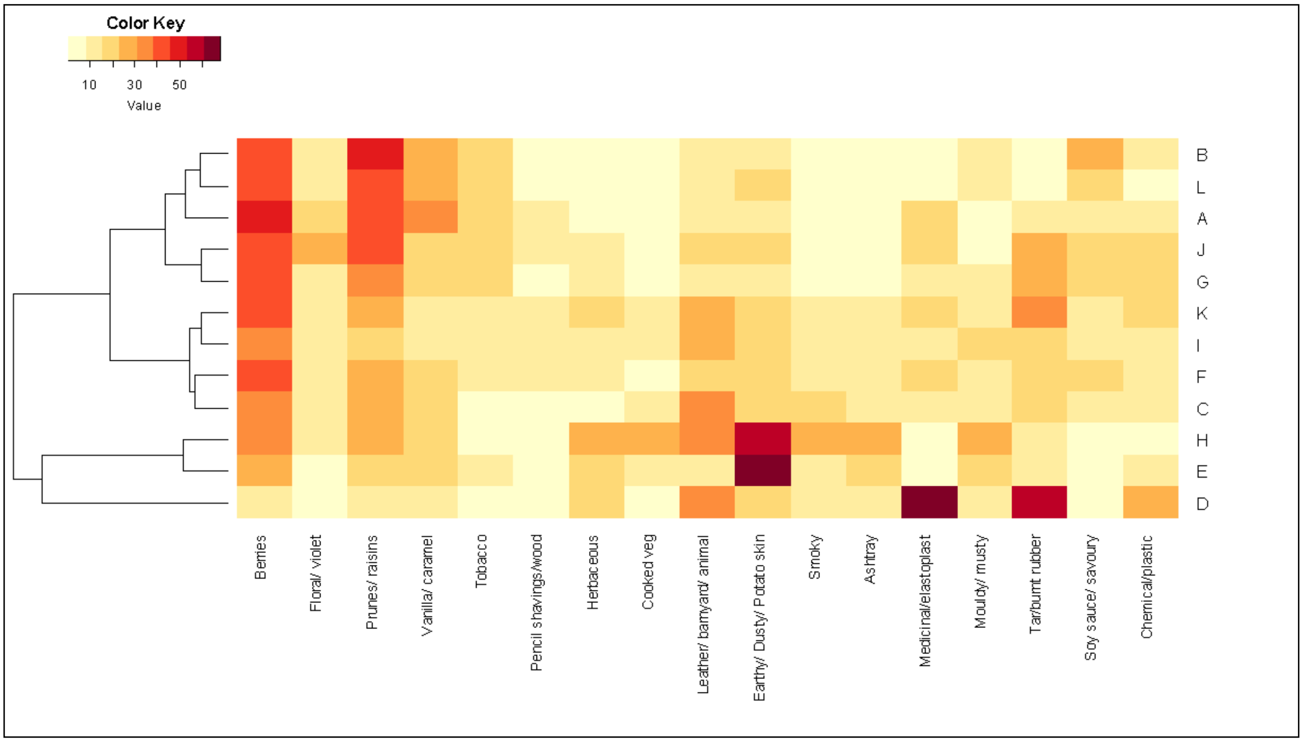


Figure 3. Heatmap generated with R using sensory numeric matrix data of seventeen aroma attributes associated with the twelve wines (A- L)

On the horizontal axis, the seventeen sensory attributes are shown. Vertically, wines A to L are presented and the differences in the wines per attribute can be seen. Color (or a shaded scheme) is used to represent 'bins' of average intensities for each attribute according to the 0-100 scale assigned by panel members. Wines are grouped in a dendogram on the left hand side of the heatmap based on a standard hierarchical clustering of similarity or dissimilarity of attributes and intensities. As can be seen, wines B, L, A, J and G are most closely associated with berry and prune flavours, and few other attributes. Wines K, I, F and C are grouped together and share lower intensity of most attributes generally, and exhibit some negative attributes like 'leather/barnyard' and 'tar/BR' at low levels. Wine D has strong intensities of negative attributes, but is in a sensory grouping with wines H and E, which are linked strongly through the 'earthy/dusty/potato skin' descriptor. These results mirror some findings from the LS means of the selected attributes (Figure 3), and the results of the PCA (Figure 5).

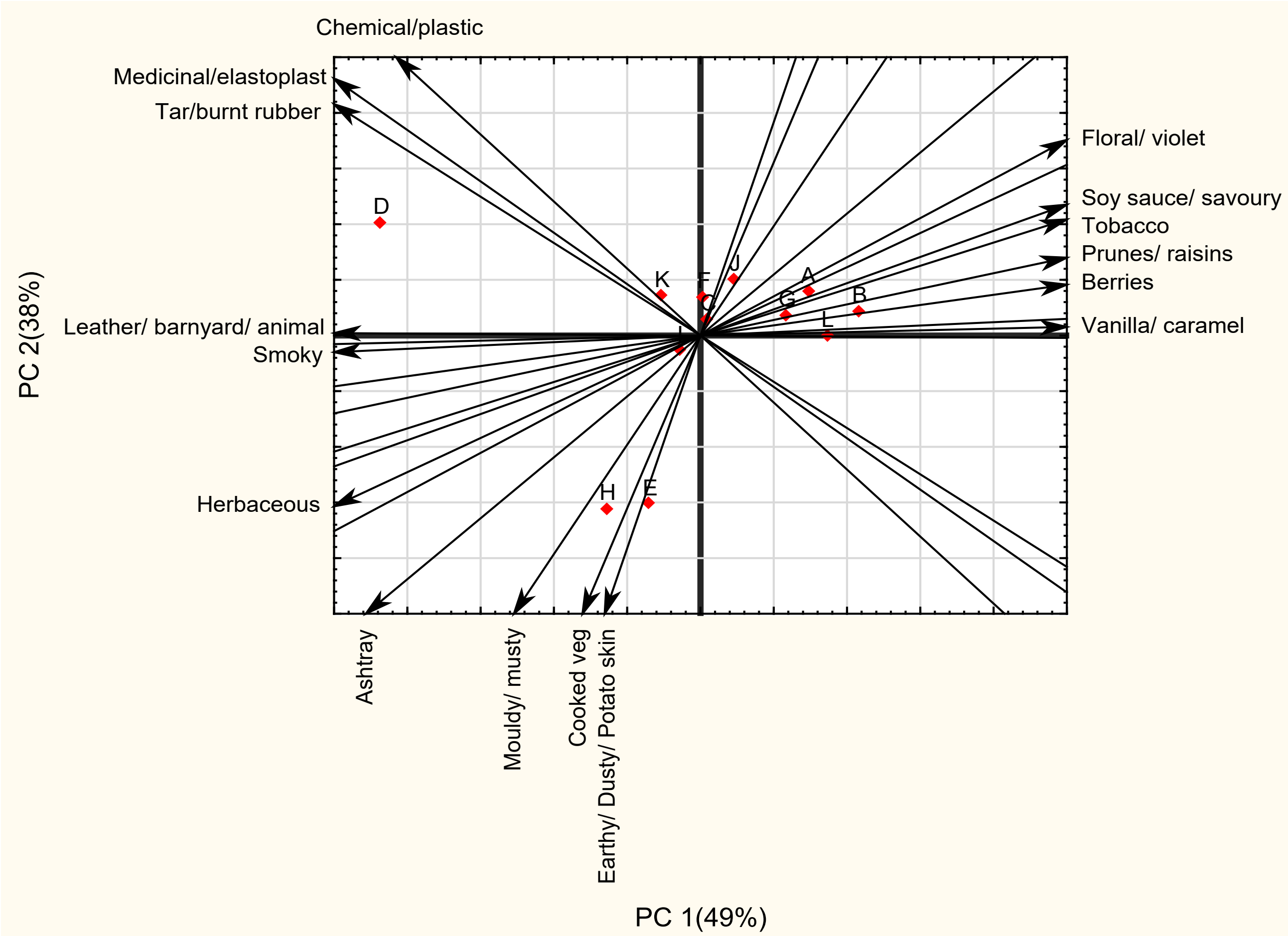


Figure 4. Principal Component Analysis prepared with Statistica from sensory data showing aroma attributes associated with wine samples A-L.

In the PCA, the first two principal components explain more than 80% of the variation in the sensory dataset. Loadings for wines J, L, K, I and C show relative groupings with wines A, B, L and G in the quadrant closest to descriptors such as ‘berries’, ‘floral/violet’, ‘prunes/raisins’ and ‘vanilla/caramel’. These two groupings (J, F, L, K, I, C and A, B, L, G) are present for both PC1/2 and PC1/3 (not shown). Wines H and E form a group that is associated with descriptors such as ‘cooked veg’, ‘mouldy/musty’ and ‘earthy’, and these wines separate on PC2. Wine D separates out most strongly from all the other wines, and is most closely associated with wines that have attributes leather/barnyard and tar/BR.

3.2 GC-MS results for VP analysis

Results for the GC-MS analysis (averages for three instrumental repeats) of VPs are listed in Table 4, which also indicates where levels of compounds exceed the ODTs commonly used in the literature.

Where possible, ODTs for red wine were used, but if not available, the ODT most appropriate to the study was considered.

As can be seen from Table 4, all of the wines contained at least one of the VPs at peri- or supra-threshold levels, and all of the wines (except B, J and L) contained guaiacol and *p*-cresol at potentially detectable levels. A few wines (C and D in particular) were notable in their very elevated levels of specific VPs. Most wines had low levels (below ODT) of the eugenol, phenol and 2,6-dimethyl phenol. Wine D is particularly high in a number of VPs. Guaiacol is present at twice odour threshold. The level of 4MG is 859 µg/L, around 40 times its ODT in water. The cresols are also found in high concentration in wine D: *m*-cresol is present at 180 µg/L, or around three times its ODT in model wine; p-cresol at 17 times its ODT in model wine (173 µg/L). The xylenols are also present at higher levels than in the other wines: 2,3-DMP almost at its ODT levels and 3,4-DMP a 681 µg/L, at around half its ODT. Significantly, 4-EP is present very near its ODT (550 µg/L).

Table 4. GC-MS Quantification of of volatile phenols in µg/L (expressed as average (SD) of three repeats)

Wine	guaiacol	2,6- DMP	4- MG	*o*- cresol	phenol	4- EG	*m*- cresol	*p*- cresol	2,3- DMP	eugenol	4- EP	3,4-DMP
A	23 (5)	7 (0.2)	12 (2.1)	28 (2.2)	10 (1.6)	2 (0.4)	36(0.2)	15 (1.3)	12 (1.2)	24 (4.1)	3 (0.8)	3 (0.2)
B	7 (1.1)	3(0.4)	7 (0.6)	14 (1.0)	5 (0.6)	2 (0.1)	25(0.9)	9 (0.5)	7 (0.8)	4 (0.3)	2(0.1)	2 (0.2)
C	61 (6.0)	11 (1.8)	26 (2.0)	317 (21)	133(23)	5 (0.2)	65(8.2)	35 (4.7)	27 (4.0)	1(0.3)	5(0.2)	6 (1.6)
D	54 (8.8)	2 (0.7)	859(81)	23 (2)	51(8.0)	3 (1.0)	180 (21)	173 (9)	406 (30)	3(0.2)	550 (47)	681 (27)
E	54 (5.3)	1 (0.5)	49 (2.0)	19 (1.3)	9 (0.7)	1 (0.2)	8 (0.8)	5(0.3)	75(6.6)	2(0.1)	174 (24)	220 (12)
F	16 (3.0)	6 (0.5)	8 (1.1)	14 (1.9)	14(1.7)	16 (1.6)	16 (1.1)	8 (0.5)	15 (2.4)	49(7.0)	22 (5.0)	24 (5.2)
G	29 (2.5)	5 (0.4)	15 (1.5)	28 (1.4)	8(0.6)	4 (1.1)	30 (0.7)	27 (3.1)	19 (3.0)	42 (5.3)	4 (0.4).	6(1.0)
H	61 (11)	1 (0.2)	54 (4.1)	15 (2.)	8(1.0)	1 (0.2)	7 (4.9)	4(1.7)	120 (18)	1(0.2)	263 (30)	315 (32)
I	23 (5.7)	7 (0.1)	20 (5.9)	97 (11)	44(3.0)	7 (0.3)	84 (17)	30 (4.9)	26 (4.3)	21 (0.2)	1.2 (0.4)	15(3.4)
J	174 (3.6)	6(0.7)	10 (1.5)	27 (2.4)	12(2.0)	4 (0.2)	20 (4.5)	9(2.9)	8 (1.0)	21	4 (0.7)	5(0.4)
K	23 (4.0)	11(2.0)	14 (2.5)	25 (3.7)	13(0.7)	9(0.2)	14 (2.9)	7(1.0)	12 (2.0)	22 (0.2)	14 (1.5)	16 (2.3)
L	20 (5.7)	7(1.5)	13 (1.1)	36 (5)	16(2.6)	2 (0.4)	32 (7.4)	12 (0.8)	9(2.0)	38 (4.1)	3(0.1)	4 (0.8)

A heatmap was also compiled for the VP data (Figure 6). As the VP data showed levels that differed by several orders of magnitude (Table 4), standard scores (z-scores) were calculated in order to standardize the data, and minimize distortions caused by different compound levels. The z-score for each compound was calculated using the formula $z = (x - \mu) / \sigma$ where x is the individual value for the compound, μ is the mean for each compound group, and σ is the standard deviation for the group. Compounds are presented on the horizontal axis, and wines A to L are presented vertically so that the differences in the wines per compound (z-score) can be viewed. As previously, color is used to represent 'bins' of average intensities for each compounds according to the VP z-scores with blue indicating levels higher than the mean (pale yellow). Wines are grouped on the left hand side of the heatmap based on a standard hierarchical clustering of similarity or dissimilarity of z-scores.

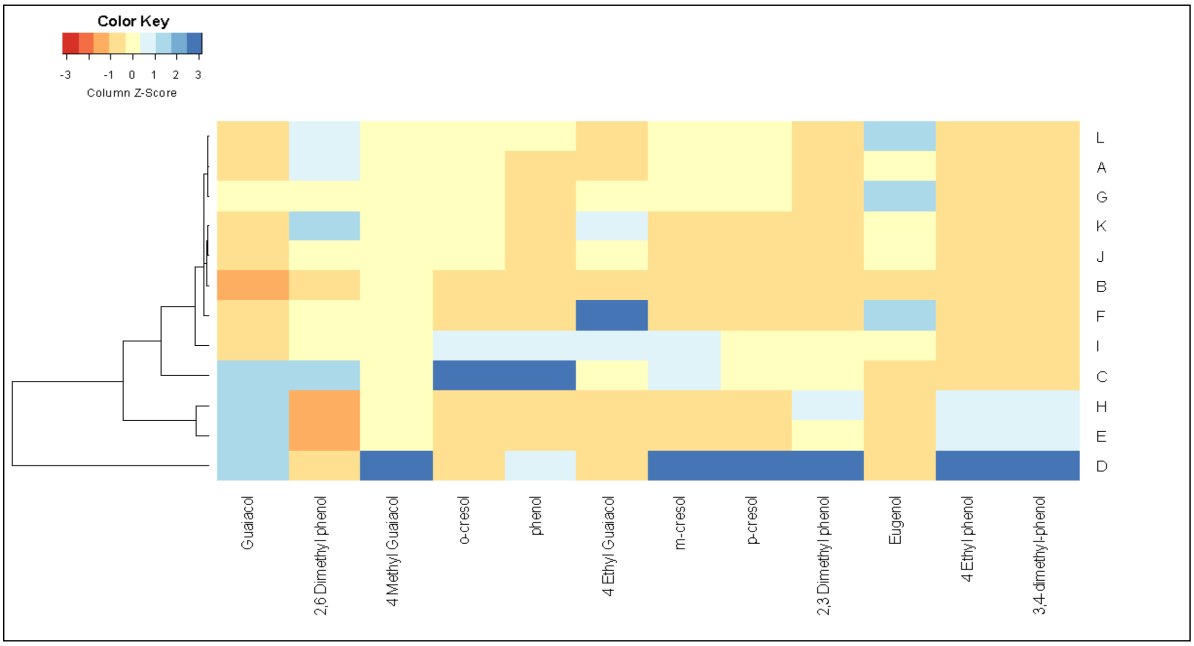


Figure 5. Chemical heatmap compiled using z-scores of GC-MS data for volatile phenols

Inspection of the chemical heatmap shows similarities in wine groupings compared to those in the heatmap of sensory attributes (Figure 4). Wines D-H-E form a grouping based on similar VP composition (notably guaiacol, 4-EP, and 3,4-DMP). Wine D separates out from this group based on much higher z-scores for most of the VPs measured, specifically 4-MG. Wines L-A-G form a grouping, very closely related to K-J-B, based on low phenolic contents, with only 2,6-DMP and eugenol for the former grouping showing z-scores slightly higher than the mean.

3.3 Combined sensory and chemical data

A multiple factor analysis (MFA) correlation plot was generated combining results for 12 VPs and 17 aroma attributes (Figure 7A). Compounds and/or attributes that contributed to the first and the second dimensions are located within the two correlation circles. Together the two dimensions account for 66.1% of the variance within the dataset. The inner circle represents a correlation factor (R^2) of 0.7 and the outer circle a correlation factor (R^2) of 1. In Figure 5A, attributes located along the positive axis of dimension 1 include ‘chemical/plastic’, ‘tar/BR’, ‘medicinal/Elastoplast’, ‘leather/barnyard’.

These attributes are associated with *p*- and *m*-cresol, 4-MG, 2,3-DMP, 4-EP and 3,4-DMP. Wine D is positioned in this region of the MFA (Figure 7B), but very much further out: the axes in the IMF (Figure 7B) have been expanded in the positive direction along dimension 1 to accommodate this wine, and along dimension 2 in the negative direction to accommodate wines E and H. Attributes located along the negative axis of dimension 1 are ‘prunes/raisins’, ‘floral/violet’ and ‘tobacco’, and most of the wines form a grouping in the negative quadrant along dimension 1 closer to these attributes and associated with eugenol, 2,6-DMP and 4-EG. The broad separation in dimension 1 therefore seems to be between sweet-associated attributes and faulty / negative attributes on the opposite side of the plot origin. Dimension 2 separates chemical–related attributes like ‘chemical/plastic’ and ‘tar/BR’ rubber and more vegetal-earthy attributes in the negative direction of this dimension. VPs associated most closely with the chemical attributes are p- and m-cresol, and 4-MG. Guaiacol is most closely associated with the ‘smoky’, ‘ashtray’ and interestingly, ‘herbaceous’. Most of the wines have sweet-associated attributes, as can be seen in the IFM (Figure 5b), but wine D is strongly separated out and associated with ‘chemical’ type faults, and wines E and H separate out towards the ‘earthy/dusty’, ‘cooked veg’ and ‘mouldy/musty’ attribute set.

It does not appear, from this dataset that *o*-cresol and phenol are associated with any of the positive or negative attributes. In the scatterplot of chemical compounds and wines (Figure 8), wine C separates out because of *o*-cresol and phenol content, which manifests in some smokiness (not significantly different from wine H) and ‘leather/barnyard’ attribute, on par with wine D.

There are a number of aromas (‘earthy/dusty/potato skin’, ‘cooked veg’, ‘mouldy/musty’ and ‘herbaceous’) on the MFA in the negative direction of both dimensions that are not explained by close association with specific VPs at peri- or supra-threshold concentrations, and which may be the result of olfactory effects of subthreshold combinations.

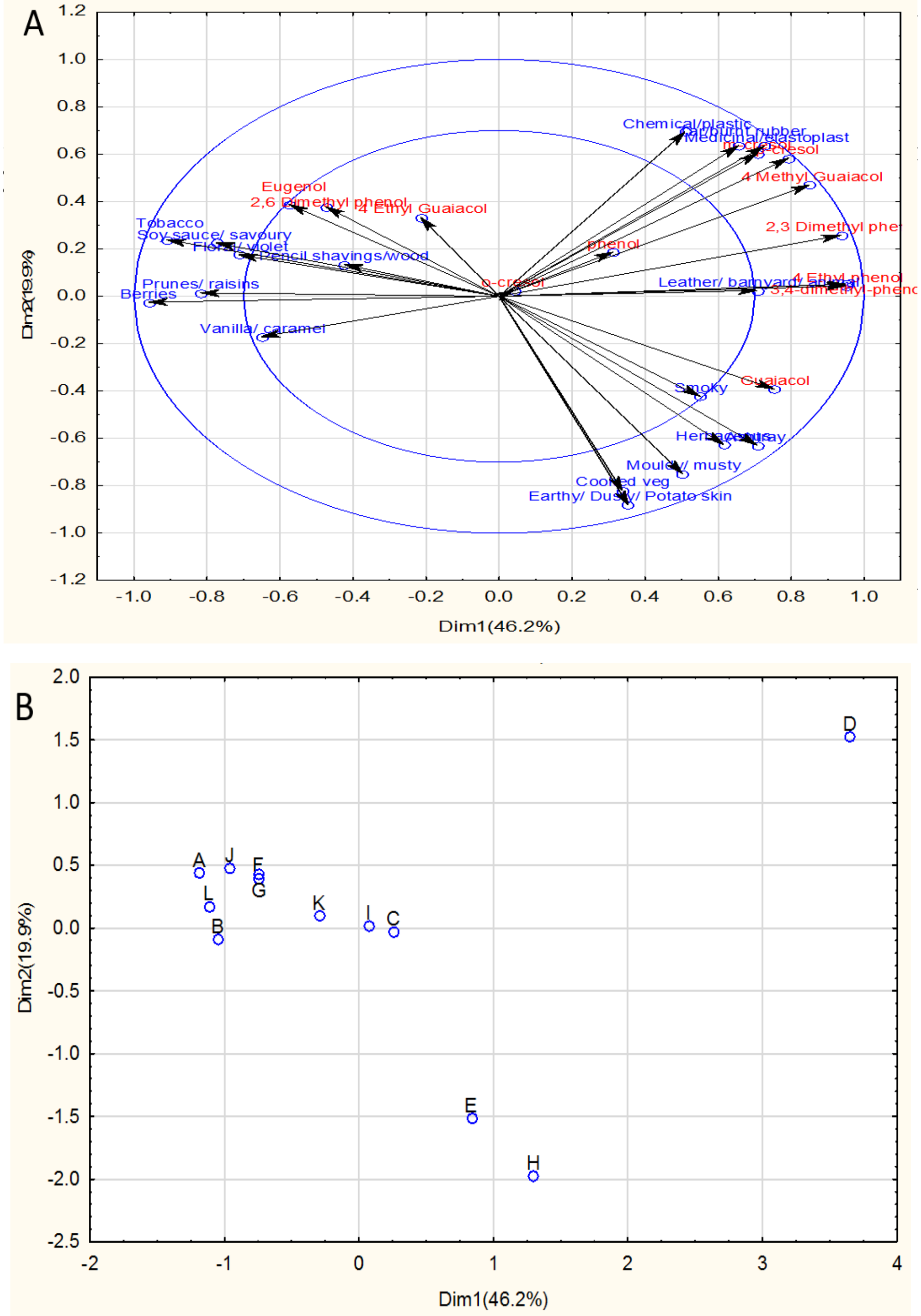


Figure 6A: Multiple Factor Analysis (MFA) correlation plot of combinations data with aroma attributes and chemical compounds shown (Dim1/Dim2). Figure 7B: individual factor map (IFM) for wines A-L.

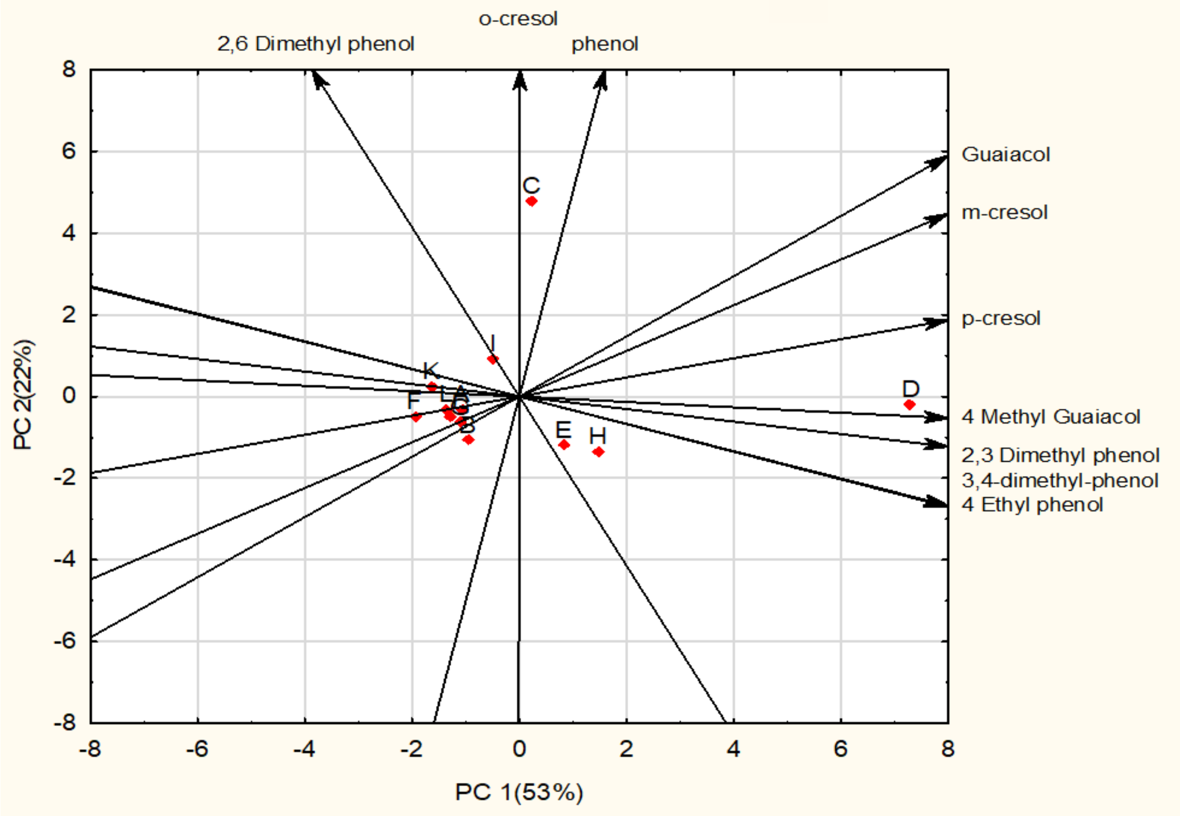


Figure 7. Principal Component Analysis (PCA) of combination data with volatile phenols shown. Sample codes represent the wine samples A-L as outlined in Table 2.

4. Discussion

Although previous research has shown the importance of VPs in smoke taint, it is crucial to consider aspects other than the VP concentrations that can impact on the aroma of wines. It is well known that the grape cultivar plays an important role in the overall aroma profile of the wine due to the presence of primary aroma components such as terpenes, methoxypyrazines and norisoprenoids (Ilc *et al.*, 2016) that migrate from the grape to the wine during the vinification process. Compounds at peri- or sub-threshold levels may have their sensory contribution merged with that of the cultivar, with subsequent masking (Hein *et al.*, 2009). Wine age has an impact on the formation of aging bouquet, and may increase levels of ethyl acetate and acetaldehyde that could have a masking or additive effect on certain components (Coetzee *et al.*, 2016). Ethanol concentration has been shown to affect the volatility of certain components (Goldner et al. 2009), with wine being described as herbaceous instead of fruity at higher alcohol levels. In the current sample set, the alcohol levels of the wines were similar, and the panel did not find that alcohol dominated in any of the aroma profiles. It is also noteworthy that the odour detection thresholds for six of the VPs analyzed are only available in the literature for water, and two are available only for alcohol (model wine) solution (Table 1). Only four of the compounds in this study have had ODT levels established in red wine, and these were not the same wine as those under analysis here, so the value of these ODTs to the current study is questionable. They can only offer a tentative guideline as to how powerful the compound's odour activity will be in these matrices. OAVs (Odour Activity Values) were not calculated for this reason.

VPs are known to be produced during bushfires (Krstic et al. 2015), absorbed by grapes (Ristic et al. 2015), and carried through to wine (Ristic et al. 2011). It was therefore of value to consider bushfire events that may have impacted grapes prior to harvest. The Western Cape in South Africa has hot, dry summers, and the natural vegetation (the *fynbos*) has evolved to burn regularly (Strydom & Savage, 2016). *Fynbos* fires are rapid and fairly cool, moving very fast over mountainous regions with the assistance of often gale-force South Easterly winds, accompanied by smoke that can cover hundreds of square kilometers. Vineyards are located all over the province, and are frequently in the path of these bushfires. It is not unlikely, therefore, that grapes will be exposed to a range of smoke-associated volatiles including VPs, which may then transfer to wine (de Vries, Mokwena, *et al.*, 2016).

Two sources were used to trawl historical data on fires in the Western Cape. These were Forest Watch (Fire) (https://fires.globalforestwatch.org/) and Advanced Fire Information Systems (AFIS) (https://southernafrica.afis.co.za/). Both websites provide detailed data on various aspects of bushfire monitoring via low Earth orbit satellite, and have historical archives relating to fire events going back to 2008 and covering most land masses, and are a very useful resource for tracking fires in real time.

Although the phenological stage of smoke events discussed below is unknown, dates for bushfire data were targeted for the typical harvesting period for red wines in the Western Cape, viz, February to April. The closer to harvest the fire event occurs, the more impact it will have on the flavour of wine made from smoke-affected grapes (Shepherd, *et al.*, 2009, Kennison *et al.*, 2009). It is acknowledged that this is a wide window, but the potential for smoke taint exists. A number of the sensory attributes may be explained in terms of the VP composition of the wines in this study, and the available ODTs for the various compounds. The PCA of the sensory results of the wines supports the frequency and intensity listings given in the heatmap and in the LS means graphs, as it shows that loading for samples separate into broad groupings. The dendogram of chemical results (Figure 4) also shows a separation according to chemistry into similar groupings.

Based on the sensory and chemical data, wines A,B,G, J and L formed a broad chemical and sensory grouping with low VP contents, and positive attributes. Sensory characterization of these wines showed high levels of sweet-associated attributes, with the 'berries' descriptor, and 'vanilla/caramel' being the attributes with the highest means. As can be seen in the sensory heatmap (Figure 4), few negative descriptors were given for this group of wines. Wines A and B were Grenache from the Franschhoek region of the Western Cape (2015 vintage and 2016 respectively), wine G was a Syrah from Elgin (2016), and Wines J (a blend) and L (Pinotage) were both vintage 2015. Based on date queries with ForestWatch and AFIS websites, these wines were all from regions that were unaffected by bushfires during the period leading up to harvest, with the exception of wine B. Fires between

Feb and April 2016 in the La Rochelle Nature Reserve, as well as near the Berg River dam in Franschhoek may have affected this wine, but it was subject to one round of reverse osmosis (RO) due to suspected smoke taint. The winemaker submitted the wine for VP analysis to check that the RO had worked, which sensory evaluation confirmed. There were no fires reported in 2015 in the Franschhoek valley during the period leading up to harvest. There were fires in the Grabouw town area between March and April 2016, which may have affected wine G, however it showed no significantly negative characteristics despite having peri-threshold levels of guaiacol and *p*-cresol. Wine producing areas in Elgin lie to the south east of Grabouw, and prevailing wind is a strong south easterly throughout summer over this region. Smoke and ash would be likely to have been carried on the wind over the mountains to the South-East towards the Helderberg basin, away from Elgin. Wine J was a Cabernet-Merlot blend from the Stellenbosch region, and most associated with the 'floral' descriptor (mean intensity of 23.77). There were no fires recorded in the Stellenbosch region in 2015, although it was a bad year for fires in other regions. Durbanville was also unaffected by bushfires in the period leading up to harvest 2015, and wine L (a Pinotage from Durbanville) also does not exhibit any strong attributes. In fact, wine L showed a tendency to be lower in negative attributes like 'tar/BR', 'medicinal/Elastoplast™' and 'cooked veg' than most of the other wines.

The second grouping of wines that is suggested by chemical and sensory data is the K,I,F group, which unlike the first group, is not associated with positive fruity descriptors. These data support the findings by Atanasova *et al*.(2005), who observed that sub- and peri- threshold concentrations of woody compounds (including guaiacol) can modify the perception of a supra-threshold fruity odour. Wine F (a 2016 Cabernet Franc from Elgin) does not have any VPs at peri- or supra- threshold levels (Table 3), and does not exhibit any strong characteristics. The descriptors with the highest means for wine K were 'tar/BR' (mean intensity 33), and 'leather/barnyard' but these were not significantly different from a number of the other wines. Wine K is WO 'Western Cape' (vintage 2016), Merlot and Cabernet Sauvignon blend, which means it the grapes may be sourced from different areas of the province. This wine contained guaiacol at peri-threshold concentration, but all other VPs were well below their ODTs. As previously noted, the Western Cape was affected by severe bushfires during 2015 and 2016, which may explain the presence of guaiacol. The wine may also have had wood maturation, as this was not specified by the producers when samples were submitted. Wine I was a Cabernet Sauvignon from the Durbanville region (vintage 2015). Despite having a number of VPs at peri- and supra-threshold level (Table 3), this wine also had no outstanding attributes. There were no notable fire events in Durbanville area during February to April 2015. Five of the VPs are present at supra-threshold levels, which would suggest that they should be detected by a trained panel, but this was not the case. The wines did express high fruit intensity, and this could well have masked any sensory contribution by the VPs present in these wines, as has been indicated by Atanasova *et al*., (2005) previously. These authors, and later De Vries *et al,* (2016) showed that guaiacol could contribute 'sweet, woody' notes to wine which cannot be considered off-flavours, but Lorrain *et al*., found that VPs could impact red wine esters (sweet, fruity notes), so the olfactory space is complex..

Additonally, the presence of other compounds like IBMP, which is known to be an important primary aroma contributor in Cabernet Sauvignon, and can affecting olfactory perception and mask other contributors (Hein *et al.*, 2009).

Wine C (a Cabernet Sauvignon from the Stellenbosch WO region, vintage 2012) shares some characteristics with the K, I, F group, but also with wines H and E. AFIS records large bushfires between February and April 2012 in the Jonkershoek region, directly due South East of Stellenbosch. Wine C had the highest levels of *o*-cresol and phenol of all the wines, which would explain the significantly higher (p=0.05) 'smoky' attribute (Figure 3-i). The 'leather/barnyard' (Figure 3-vi, mean intensity 33.03) was higher than all other wines except H. This attribute is interesting because it is normally associated with 4-EP, and this wine contains negligible levels of this compound. The 'leather' characteristics may be due to olfactory effects of the cresol and phenol with other compounds, including IBMP, have been described before (Lorrain *et al*. 2013b, Campo *et al*. 2005).

Wines E (Cabernet Franc) and H are strongly associated with negative attributes (Figures 3). Wine E was a Cabernet Sauvignon from Franschoek (2016), and was significantly higher (p=0.05) in 'earthy/dusty/potato skin' 'mouldy/musty' and 'ashtray' attributes. Fires between Feb and April 2016 in Franschhoek may have affected this wine. As Franschhoek lies in a valley between high mountain peaks, smoke could have been trapped in the in low-lying areas and affected grapes in the period leading up to harvest. As this is also Cabernet Sauvignon, it is possible that the 'earthy/dusty/potato skin' could have been the result of supra-threshold levels of guaiacol and 4-MG interacting with IBMP and causing olfactory effects.

Wine H (Merlot) was also significantly higher (p=0.05) in the 'earthy/dusty/potato skin' (mean intensity 60.45) attribute than all the other wines (Figure 3). There were a great many large bushfires during March-April 2015 all over the Western Cape, but particularly bad fires in the Helderberg region, with smoke trapped for several days in the Helderberg basin. Fires burned for days in the Steenbras area, prevailing wind South East, taking huge quantities of smoke and ash into Helderberg valley and wine producing areas. Wine H is significantly higher in the ashtray attribute (Figure 3ii), and shows 'green' characteristics as it is significantly higher in 'cooked veg' attribute (Figure 3iii) and the 'herbaceous attribute (Figure 3iv), both of which are associated with the cultivar. This wine also is one of the highest in 'leather/barnyard' aroma.   In wine samples E and H, guaiacol and 4-MG are present at supra-threshold levels, and 4-EP and 3,4-DMP are at approximately half their literature threshold values. The MFA (Figure 7) indicates that 4-EP and 3,4-DMP are associated with 'leather/barnyard/animal' attributes. Guaiacol is associated strongly with the 'smoky' and 'ashtray' attributes (Figure 7), but also, interestingly, is also close to the 'herbaceous' and 'mouldy/musty' attributes. In the MFA (Figure 7), *o*- and *p*-cresol, as well as 4-MG and 2,3-DMP are associated with 'chemical/plastic', 'tar/burnt rubber' and 'medicinal/Elastoplast™' attributes. The wines were submitted as definitely or potentially smoke-tainted by industry, and it may be that the mouldy, leathery or herbaceous characteristics could have added to, or been mistaken for smoke-taint by industry members not specifically trained in identifying smoke-taint attributes.

Wine D was characterized by intense negative attributes, and high VP content and separated out in both sensory and chemistry results from other samples. The wine is characterized by 'medicinal/ Elastoplast™' (mean intensity 68.53), 'tar/BR' (60.87) and chemical plastic (30.47), all significantly higher than other wines (Figures 1 and 3). The 'leather/barnyard' (37.87) attribute was also higher than most of the other wines. This wine is from the De Doorns region, vintage 2014. It is a Cabernet Sauvignon, a cultivar that is traditionally harvested fairly late in the season. March-April data for 2014 from the AFIS system shows bushfires on the slopes of the mountains directly to the south, and close to the town. As the WO area is in a long, deep valley running approximately north to south, with wine and grape growing areas spread across the bottom of the valley, it is entirely conceivable that smoke settled in the valley, and was absorbed by grapes prior to winemaking. Previous work in this area of research has suggested that combinations of VPs can cause a 'burnt rubber' or 'tar' attribute (Panzeri, 2013), as seems to be the case in the last wine sample D. As most of these wines were submitted for assessment for smoke taint, it is likely that this wine was made from grapes affected by bushfires in the De Doorns region.

Despite this, the levels of VPs in the samples, specifically 4-methylguaiacol, *o*- and *p*-cresol and 4-ethylphenol, are not always consistent with wines made from grapes that have been been exposed to natural wildfires where guaiacol and syringol are normally elevated (Hayasaka, *et al*.,2010; Parker *et al*., 2013; Krstic, 2015) . It may well be that the VP levels in wines D, and E, (given the elevated levels of 4-EP), could be due to other possible sources such as Brettanomyces or oakwood contact and/or contamination of these compounds from other sources. It could also be the case that wines E and H (Merlot and Cabernet Sauvignon) are reflecting varietal character in their 'green' notes.

5. Conclusions

This study investigated the levels of volatile phenols found in South African red wines that had been selected by industry as actually or potentially smoke tainted. VP chemistry and sensory results showed that wines could be broadly divided into four groupings, using chemical and sensory indicators. VP content of samples could be correlated, in most but not all of the cases, to sensory descriptors for the wines, when odour detection thresholds for the compounds were taken into account. It was demonstrated that certain sensory attributes ('smoky', 'ashtray') in some of the wines could be ascribed to higher levels of specific, or combinations of, VPs at peri-threshold levels. In other cases, it appeared that combinations of compounds (for example, cresols and xylenols) at subthreshold levels led to unexpected sensory effects ('earthy/dusty', 'chemical' and 'tar/burnt rubber'). Guaiacol was present in the majority of samples at or above ODT, but as the wines had been submitted by industry for suspected, or perceived smoke taint, this result was not surprising. Also, whether samples had received any oak treatment was a crucial omission in information provided on the samples, as wood treatment is a well-known source of this compound. However, guaiacol did not seem to be correlated with a perception of ‘smoke’ in any of the wines unless it was in combination with other phenols, and in fact may have contributed to sweet-associated and fruity aromas in the majority of samples. It is notable that out of twelve wines, the four (C, H, E and D) that were described with the most negative attributes, at significantly higher levels than the others, were all from regions that had experienced severe fire events. Out of eight wines that did not show negative attributes, only two were from regions that had experienced bushfires in the period leading up to harvest, and one of these had been treated with reverse osmosis.

It may be the case that a prescreening of the samples by expert tasters in smoke taint may have established that a number of the wines were not affected by smoke taint, negating the need for full sensory evaluation and analysis. However, this requires that industry and/or researchers are trained to a high level. The subsequent investigation and discussion highlights the fact that these issues are more complex than smoke exposure of grapes causing smoke taint in wine, and the uncertainty around this type of information. There is certainly a need for better methods for monitoring smoke exposure in wine regions.

This study also emphasizes the importance of understanding the effects of compounds like VPs on wine, and escalating awareness of, and sensitivity to, the interactions and synergies between them. Further research through carefully considered experiments would help to clarify effects of compounds at various levels and in different matrices. It would also be useful to establish or confirm odour detection thresholds in specific matrices, as there seems to be limited information published in this regard. There is also value in investigating amelioration of the sensory effects of VPs if they are prominent and negatively impact on wine quality.

Acknowledgments

Prof. Martin Kidd of Stellenbosch University is acknowledged for analyzing the statistical aspects of the study. Winetech and THRIP (NRF) acknowledged funding associated with volatile phenol projects.

Author disclosures: Marianne McKay, Astrid Buica and Florian Bauer conceived and designed this study; Lucky Mokwena performed the GC-MS volatile phenol analyses. Valeria Panzeri led the sensory evaluation panel. Marianne McKay acquired the funding, carried out the experimental work and wrote the paper, which was revised and edited by all the authors.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

Atanasova, B., Thomas-Danguin, T., *et al*., 2004. Perceptual interactions between fruity and woody notes of wine. Flavour Fragr. J. 19, 6, 476–482.

Barnes, T., 2018. Portugal wildfires The Independent. (London) 5 August: News/World/Europe.

Boidron, J.N., Chatonnet, P., *et al*., 1988. Influence du bois sur certaines substances odorantes des vins Connaiss. la Vigne du Vin 22, 4, 275–294.

Campo, E., Ferreira, V., *et al*., 2005. Prediction of the Wine Sensory Properties Related to Grape Variety from Dynamic-Headspace Gas Chromatography−Olfactometry Data. J. Agric. Food Chem. 53, 14, 5682–5690.

Campo, E., Ballester, J., *et al*., 2010. Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: An application to Burgundy Pinot Noir wines. Food Qual. Prefer. 21, 1, 44–55.

Coetzee, C., Brand, J., *et al*., 2015. Sensory interaction between 3-mercaptohexan-1-ol, 3-isobutyl-2-methoxypyrazine and oxidation-related compounds. Aust. J. Grape Wine Res. 21, 2, 179–188.

Coetzee, C., Brand, J., *et al*., 2016. Sensory effect of acetaldehyde on the perception of 3-mercaptohexan-1-ol and 3-isobutyl-2-methoxypyrazine. Aust. J. Grape Wine Res. 22, 2, 197–204.

Cox, L., 2018. Sydney's bushfire season starts in winter: "We may have to rethink how we live" Guard. Int. Ed. (Sydney, Australia) https://www.theguardian.com/cities/2018/aug/15/syd.

Goldner, M.C. Zamore, M., Di Leo Lira, P., *et al*., 2009. Effects of ethanol level in the perception of aroma attributes and the volatile composition of red wines. J. Sens. Stud. 24, 2, 243–257.

Goode, J., 2008. Burnt Rubber: The great South African wine debate. Available at http://www.wineanorak.com/blog/2008/10/burnt-rubber-great-south-african-wine.html.

Hammond, C.E., 2015. South African Wine Under Fire Available at http://www.carolynevanshammond.com/blog/2015/10/12/south-african-wine-under-fire-1.

Hein, K., Ebeler, S., *et al*., 2009. Perception of fruity and vegetative aromas in red wine. J. Sens. Stud. 24, 3, 441–455.

Heyns, E., 2014. The Green South African palate - When does mint become eucalyptus or even downright weedy? - Wineland Magazine Available at http://www.wineland.co.za/the-green-south-african-palate-when-does-mint-become-eucalyptus-or-even-downright-weedy/.

Ilc, T., Werck-Reichhart, D., *et al*., 2016. Meta-Analysis of the Core Aroma Components of Grape and Wine Aroma. Front. Plant Sci. 7, September, 1–15.

Jin, Y., Goulden, M.L., *et al*., 2015. Identification of two distinct fire regimes in Southern California: implications for economic impact and future change. Environ. Res. Lett. 10, 9, 094005.

Kennison, K., 2013. Effect of smoke in grape and wine production. Gov. West. Aust. Dep. Agric. Food Bull. Bulletin 4.

Kennison, K.R., Wilkinson, K.L., *et al*., 2009. Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Aust. J. Grape Wine Res. 15, 228–237.

Kennison, K.R., Wilkinson, K.L., *et al*., 2011. Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties. Aust. J. Grape Wine Res. 17, 2, 5–12.

Krstic, M., Johnson, D., et al., 2015. Review of smoke taint in wine: Smoke-derived volatile phenols and their glycosidic metabolites in grapes and vines as biomarkers for smoke exposure and their role in the sensory perception of smoke taint. Aust. J. Grape Wine Res. 21, 537–553.

Lapalus, E., Wessel, P., *et al.*, 2016. Linking sensory attributes to selected aroma compounds in South African Cabernet Sauvignon wines, Master's Thesis, Stellenbosch University.

Lorrain, B., Tempere, S., *et al.,* 2013. Influence of phenolic compounds on the sensorial perception and volatility of red wine esters in model solution: An insight at the molecular level Food Chem. 140, 1–2, 76–82.

Margalit, Y. 2013 Concepts in Wine Chemistry, Publisher Wine Appreciation Guild, Publication City/Country South San Francisco, United States. ISBN13 9781935879817

Panzeri, V., 2013. Influence of vineyard posts type on the chemical and sensorial composition of Sauvignon blanc and Merlot noir wines. Master's Thesis, Stellenbosch University

Parker, B.M., Baldock, G., *et al.*, 2013. Seeing through smoke. Wine Vitic. J. 42–46.

Perez-Llamas, C. & Lopez-Bigas, N., 2011. Gitools: Analysis and Visualisation of Genomic Data Using Interactive Heat-Maps. PLoS One,Vol 6, No. 5, e19541 p1-6.

Petrozziello, M., Asproudi, A., *et al.*, 2014. Influence of the matrix composition on the volatility and sensory perception of 4-ethylphenol and 4-ethylguaiacol in model wine solutions. Food Chem. 149, 197–202.

Ristic, R., Van Der Hulst, L., *et al.*, 2017. Impact of Bottle Aging on Smoke-Tainted Wines from Different Grape Cultivars. J. Agric. Food Chem. 65, 20, 4146–4152.

Romano, A., Perello, M.C., *et al*., 2009. Sensory and analytical re-evaluation of “Brett character”. Food Chem. 114, 1, 15–19.

Sheppard, S., . Dhesi, M. *et al*., 2009. Effect of Pre- and Postveraison Smoke Exposure on Guaiacol and 4-Methylguaiacol Concentration in Mature Grapes. Am. J. . Enol.Vitic. 60(1):98-103

Strydom, S. & Savage, M.J., 2016. A spatio-temporal analysis of fires in South Africa. S. Afric. J. Sci. J Sci 112, 11, 1–8.

Tempere, S., Schaaper, M.H., *et al*., 2017. Masking of Several Olfactory Notes by Infra-threshold Concentrations of 2,4,6-Trichloroanisole. Chemosens. Percept. 10, 3, 69–80.

Verschueren, K., 2009. Handbook of environmental data on organic chemicals. John Wiley & Sons.

de Vries, C.J., Buica, A., *et al.*, 2016a. The Impact of Smoke From Vegetation Fires on Sensory Characteristics of Cabernet Sauvignon Wines Made From Affected Grapes. S. Afric. J. Enol. Vitic. 37, 1, 22–30.

de Vries, C.J., Mokwena, L.M., *et al*., 2016b. Determination of Volatile Phenol in Cabernet Sauvignon Wines, Made from Smoke-affected Grapes, by using HS-SPME GC-MS. S. Afr. J. Enol. Vitic. 37, 1, 15–21.

Wilkinson, K.L., Ristic, R., *et al*., 2011. Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine. Aust. J. Grape Wine Res. 17, S22–S28.

Wilson, C.L., 2017. Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines. Master's Thesis, Stellenbosch University.

van Wyngaard, E., Brand, J., *et al*., 2014. Sensory interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatised Sauvignon Blanc wine. Aust. J. Grape Wine Res. 20, 2, 178–185.

Chapter 4

Testing the Sensitivity of Potential Panelists for Wine Taint Compounds Using a Simplified Sensory Strategy

This article has been published in Foods **2018**, 7, 176; doi:10.3390/foods7110176 foods

MDPI

Article

Testing the Sensitivity of Potential Panelists for Wine Taint Compounds Using a Simplified Sensory Strategy

Marianne McKay [1,*], Florian F. Bauer [1], Valeria Panzeri [2] and Astrid Buica [1]

[1] Department of Viticulture and Oenology, Stellenbosch University, Stellenbosch 7600, South Africa; fb2@sun.ac.za (F.F.B); abuica@sun.ac.za (A.B.)
[2] Institute for Grape and Wine Sciences, Stellenbosch University, Stellenbosch 7600, South Africa; panzeri@sun.ac.za
* Correspondence: marianne@sun.ac.za; Tel.: +27-21-8083774

Received: 18 September 2018; Accepted: 23 October 2018; Published: 24 October 2018

check for updates

Abstract: The odor detection threshold (ODT) of a compound is the lowest concentration at which individuals can reliably perceive a difference between a sample and its corresponding control, with 50% performance above chance. Wine is a complex matrix, and ODTs used in studies on wine can be based on inappropriate matrices and informal sensory methodologies. Formal studies confirming ODTs in wine are relatively scarce in the literature, and are complex and expensive to carry out. In this study, the sensitivity of panelists to previously published ODTs for five compounds: Guaiacol, *o*-cresol and 4-ethyl phenol, 3-isobutyl-2-methoxypyrazine (IBMP), and 2,4,6-trichloroanisole (TCA) associated with off-flavor/taint issues in wine, was investigated. The study was carried out in partially de-aromatized young Shiraz wine (unwooded) using a simplified version of the formal sensory approach. A triangle test in triplicate was carried out with 34 panelists, at the ODT for each compound, in one day. The study explored whether previous training affected panelists' sensitivity for threshold differences. Results showed that samples spiked with volatile phenols were significantly different (p = 0.01) to controls. The spiked TCA and IBMP samples were not significantly different from the control in either case. Judges were better able to detect compounds if they had prior experience or training in wine evaluation. Despite some limitations, this pragmatic approach may be useful when carrying out sensory studies with fairly limited resources and within tight timelines, as it provides helpful information on panel members and detection thresholds for a specific matrix.

Keywords: odor detection threshold; wine; volatile phenol; 3-isobutyl-2-methoxypyrazine; 2,4,6-trichloroanisole

1. Introduction

Wine quality is difficult to define, but can be evaluated based on the sensory characteristics of the product using the sensory skills of experts, which include, but are not limited to, vision (clarity and color), gustation (taste), and olfaction (aroma). Wine aroma may be the most important factor in assigning quality, as faults, like microbial contamination and lack of typicality of style, are often detected by olfaction [1]. Frequently used indicators of the potency of aroma compounds associated with these faults include odor detection threshold (ODT) and odor activity value (OAV) measurements. Sensory thresholds are ill-defined in theory [2], but are based on the lowest concentration of a compound at which individuals can perceive a difference in sensory quality, relative to a control.

The ODT, which may be mg/L (ppm), µg/L (ppb), or ng/L (ppt), is numerically equal to the minimum concentration at which 50% of the judges succeed in differentiating a sample from a control [3]. The triangle test has been widely used for determining the detection threshold of volatile compounds important to wine aroma, including: Diacetyl [4]; oak lactone [5]; 3-isopropyl-2-methoxypyrazine [6], and rotundone [7]. The International Standards Organization (ISO) 13301 standard (encompassed in the American Society for Testing and Materials (ASTM) Standard Practice E679-04 [8]) has been used to establish detection thresholds for 2,4,6-trichloroanisole (TCA) [9] and ethyl hexanoate in solution with tannin and mucin [10]. The OAV is defined as the concentration of a compound present in a matrix divided by the ODT for that compound in that specific matrix. Generally, the larger the OAV, the more likely that compound would contribute to the overall odor of a complex odor mixture [10]. In theory, ODTs and OAVs may be determined this way for compounds of interest [10], but, in practice, a large number of issues influence the perception of these measures. Matrix effects, for example, have been shown to influence ODT perception in wine [11–13], and would therefore influence OAVs. The presence of other compounds in solution can also change the perception of target aromas, not just above the ODT (supra-threshold), but also below it (i.e., at sub- or infra-threshold). For example, the impact of infra- and supra-threshold concentrations of ethylphenols on wine was investigated [14], and it was found that both sub- and supraliminal concentrations of off-flavors not only changed the "hedonic valence" (positive or negative character) of the perception, but had a masking effect on fruity notes.

The composition of the panel used will also influence the success of sensory threshold studies. A panelist's ability to detect an odor will vary depending on their level of fatigue, health status, and matrix effects in the study medium [8]. The reliability of the results can be increased by enlarging the panel and by replicating tests, but this will not negate matrix effects in another medium. Due to the unique nature of the product, sensory studies on aroma in wine are very specific to particular cultivars and styles, and frequently suffer from a lack of repeatability. For example, if research on the ODT of 4-ethylcatechol is reviewed, Larcher et al. [15] determined a detection threshold of between 100 and 400 µg/L in white and red wine, while another study determined a considerably higher odor threshold of 774 µg/L [16]. The Australian Wine Research Institute noted that the odor threshold of this compound increased to 1131 µg/L in "green" red wine, and 1528 µg/L in oaky red wine [16]. It would therefore seem obvious that ODTs are confirmed in the matrix in which a study is to be carried out, but evidence for confirmation is lacking in the literature.

In this study, five odor-active compounds were selected that had been associated with specific taint issues in wine aroma, namely guaiacol, *o*-cresol, 4-ethylphenol (4EP), 3-isobutyl-2-methoxypyrazine (IBMP), and 2,4,6-trichloroanisole (TCA). Aroma descriptors for these compounds cover a continuum from "burnt, smoky" and "medicinal" to the "moldy, dusty" flavors associated with cork taint (TCA), and "herbaceous, green" aromas associated with IBMP. All of these attributes have been associated with South African red wines [13,17–20]. In order to progress with work on interactions between these compounds in a partially de-aromatized red wine, it was necessary to confirm odor thresholds that had been previously determined (Table 1), and test sensitivity of potential panelists.

Table 1. Odor Detection Thresholds (µg/L) and descriptors for compounds in red wine.

Compound	Odor Detection Threshold	Descriptors	Reference
Guaiacol	23 µg/L	Burnt, smoky, toasty phenolic	[21]
***o*-Cresol**	62 µg/L	Burnt smoky, medicinal, tar	[22]
4EP	605 µg/L	Leather, bacon, medicinal, horse	[23]
IBMP	15 ng/L	Bell pepper, green, herbaceous	[24]
TCA	4 ng/L	Moldy, musty, damp cardboard	[25]

Guaiacol exhibited the lowest odor detection threshold of all the phenols tested in a red wine matrix by Boidron et al. [26]. It forms part of the "woody" family of descriptors [22], and is extracted from pyrolyzed (toasted) oak during wood maturation of wine. The detection threshold of 23 µg/L for guaiacol confirmed by Parker et al. [21] was chosen as it was determined in a relatively recent formal sensory study at the Australian Wine Research Institute in an Australian "base red wine".

It is very difficult to find any sensory information in the primary literature about the cresols. There has been some work done in whisky, but the only current wine-related references concern its appearance in wine after smoke-events in the vineyard [27]. Boidron et al. [26] described *o*-cresol as "tar" (bitumen)-smelling, and gave an ODT of 0.8 mg/L. As the cited study was investigating wooded wines, and *o*-cresol is a known pyrolysis product of oak wood [28], this relatively high ODT seems reasonable. Parker et al. [22] reported a threshold, through a formal sensory process with 22 assessors, of 62 μg/L (standard error = 0.8) for *o*-cresol in "base red" wine. Due to the rubbery, tarry, and phenolic character of the compound, it was included in our study, and Parker's [22] threshold used, as it is the only recent threshold available in the literature carried out in an unwooded base wine.

Associated with "brettiness" and "medicinal, phenolic" smells, as well as those of "leather/horse" and "bacon/meatiness", 4EP has complex effects in wine. The detection threshold of 605 μg/L in red wine determined by Chatonnet et al. [23] is commonly cited. According to Escudero et al. [29], 4EP falls in the same semantic category as woody odorants, and in wooded wines, the wood character may mask the aroma character of the compound, making it more difficult to detect as a specific attribute (leather/horse/medicinal). This is in agreement with the findings of Curtin et al. [16], and seems to explain the high threshold found by Boidron et al. [26]. For the purposes of this study, Boidron's published detection threshold was used, as it has been cited by a number of other researchers, and is based on recognized sensory methodology.

IBMP is a potent aroma-active compound often found at higher, above-thresholds concentrations that are detrimental to red wine quality [24,30] due to its "green/herbaceous/bell pepper" characteristic. Information on the determination of odor thresholds for pyrazines in the literature is limited. IBMP, specifically, is a very strong-smelling compound in its pure form, and its odor is pervasive and difficult to disperse. It is also known to cause skin, eye, and respiratory irritation [31]. Shibamoto [32] determined odor thresholds of 46 pyrazines in water, but did not test IBMP. French studies showed that IBMP was the main contributor to vegetal aroma in red Bordeaux and Loire wines from different vintages and cultivars [24], and the ODT was determined by comparing IBMP concentrations with the intensity of the green bell pepper character. Through this, the threshold value, which seems to be a recognition threshold, and not a perception threshold, was estimated to be 15 ng/L. This does not seem to have been confirmed by any further formal sensory studies.

TCA is a compound associated strongly with cork taint in wines and is described as having a "moldy" and "damp cardboard" odor. Tempere et al. [33] noted that amongst wine defects, 2,4,6-trichloroanisole has a specific impact on wine perception. In addition to giving to the wine an unpleasant odor, it has a strong masking effect on fruity notes. It is difficult to find information on formal sensory detection threshold determinations of TCA, but in a study on the effects of TCA on consumers' wine perception [25], a level of 0.13 ng/L TCA was considered "low" and 5 ng/L was considered "high" based on concentrations frequently found in contaminated red wines. TCA detection threshold depends on the wine organoleptic characteristics, and the person perceiving it, as wine professionals had very varying abilities towards TCA detection in wine. These authors tested detection of TCA with a panel selected based on their sensitivity to this compound, in a range of red and white wines, some of which were wooded. They found TCA was detected, in red wines, at 10–15 ng/L. Prescott et al. [34] established "consumer rejection thresholds" of TCA to be 3.1 ng/L and 3.7 ng/L in Chardonnay, depending on the panel. Although Mazzoleni et al. [9] found much higher levels for Italian red wines, and Cravero et al. [35] gave an identification threshold of 7 ng/L (in Barolo), the wine in this study was unwooded and partially de-aromatized, and therefore the detection threshold of 4 ng/L, as indicated by Prescott, was chosen as suitable for the matrix.

The formal procedures for ODT determination are outlined in standard methods, specifically ASTM E679 (Determination of Odor and Taste Thresholds By Ascending Forced-Choice (AFC) Concentration Series Method of Limits) [8]. Standard practice for defining and calculating individual and group sensory thresholds are outlined in ASTM E1432-04 [3]. Normally, individual threshold calculations would require 20 to 40 AFC presentations per panelist, with data sets taken at five or more concentration scale steps (typically six or seven), with pretesting to ensure individual thresholds fall within the testing range. Obviously, this is laborious, and as the ASTM standard itself states, the costs and availability of panelists places serious limitations on what can be covered by experimenters who, typically, are limited to panels of five to 15 individuals. Even with a limited panel, a simple threshold determination will involve 100–600 presentations, which would not be achievable for most researchers. This study looked at a simple, pragmatic approach to ODT sensitivity testing for the purposes of selecting panelists for a study.

From Table 1 and the discussion above, it is clear that ODTs found in water are not comparable to those determined in ethanol solution, and thresholds in white wine are different to those found in red wine. Differences between ODTs for various compounds within the categories of red wines (different cultivars [36], wooded/unwooded [29], for example) have also been shown to exist. Even on the rare occasions when thresholds are properly determined in wine according to the official E-679 method, they are affected by the specific wine matrix and style [11,37,38], and therefore do not necessarily have relevance in other matrices. It is also evident from the literature that different people have varying responses to compounds based on their culture, experience, and age, and that training may make a difference to a person's ability to perceive an aroma [39–41].

For these reasons, the ODTs of compounds of interest to our work were investigated in a de-aromatized red wine matrix, which we planned to use in interaction studies. The sensitivity of potential panelist to compounds at ODT level was tested using a simplified version (three sets of samples per compound at the ODT) of the ASTM E-679-04 method. This study should thus provide an interesting perspective to researchers when they consider confirmation of ODTs in other matrices, as this is a pragmatic approach given the fairly complex and time-consuming standard practice. This research also set out to establish if panelists had particular sensitivities to compounds belonging to diverse aroma families, and whether previous training in sensory evaluation of wine influenced their ability to distinguish threshold differences between samples and controls.

2. Materials and Methods

2.1. Base Wine

A 2016 Shiraz wine (300 L) was supplied by a local wine producer (Koelenhof Cellar Ltd., Simonsberg, South Africa) and stored at 4 °C in 25 L food-grade plastic containers under nitrogen at the Department of Viticulture and Oenology, Stellenbosch University, South Africa. The wine had a pH of 3.6, and an alcohol concentration of 13% as determined by the supplier, who confirmed that the wine had not been treated with wood at any time during the winemaking process. Informal benchtop screening by five experienced sensory judges with tested sensitivity for the aroma compounds used to spike the wines confirmed that those compounds were not present in the base wine. The wine had an odor profile that was dominated strongly by fruit and berry aromas. This warranted partial de-aromatization following the method outlined by Wilson et al. (2017) [42] prior to threshold testing and investigations into sub-threshold interactions. The wine was de-aromatized by mixing thoroughly with activated charcoal powder (Merck, Darmstadt, Germany) for 12 h without agitation, then separated from the charcoal by diatomaceous earth filtration. During the treatment and blending steps, the wine was protected from oxidation under nitrogen gas. In a screening session, the expert panel chose a blend of 50:50 charcoal-treated wine to untreated wine, which yielded a neutral wine base with low aromatic intensity. Samples of the blend (50 mL) were taken to determine the baseline levels of the compounds investigated in this study. Analysis of volatile phenols in the de-aromatized wine was performed following the method outlined by De Vries et al. [43] using an Agilent Gas Chromatograph, model 6890N (Agilent, Palo Alto, CA, USA), coupled to an Agilent Mass Spectrometer 5975 B Inert XL EI/CI (Agilent, Palo Alto, CA, USA). Three technical repeats were analyzed for 13 volatile phenols (guaiacol; 2,6-dimethylphenol; 4-methylguaiacol; *o*-cresol; phenol; 4-ethylguaiacol; *m*-cresol; *p*-cresol;

2,3-dimethylphenol; eugenol; 4-ethylphenol; 4-vinylguaiacol; and 3,4-dimethylphenol). The base wine was found to contain very low or undetectable levels of any of the volatile phenols, including the phenols of interest. The guaiacol level in the base wine was 1.37 μg/L, *o*-cresol was 0.08 μg/L, and 4-ethylphenol concentration was 1.4 μg/L. The wine was also deemed, during informal tasting by the experienced sensory judges, to be completely free of any form of "moldy" or "herbaceous" odors that might have been associated with IBMP or TCA contamination.

2.2. Preparation of Spiked Wine Samples

The ODT levels chosen for the study were those that had been established by formal sensory methodologies, and/or were the most commonly used in literature for red wine (Table 1). These were 23 μg/L for guaiacol, 62 μg/L for *o*-cresol, 605 μg/L for 4EP, 15 ng/L for IBMP, and 4 ng/L for TCA. Stock solutions of 1000 mg/L of the five compounds were prepared in ethanol $\geq$ 99.8%, (Sigma-Aldrich, St. Louis, Missouri, United States). Guaiacol (99.3% purity), 4EP (99.5% purity), *o*-cresol (99%), IBMP, and TCA (also both 99%, Merck, Darmstadt, Germany. The compounds were dissolved in ethanol (10 mL) and then made up to volume with ultra-pure distilled water (Millipore, Bedford, MA, USA) to the concentrations required for spiking, i.e., 100 mg/L for *o*-cresol and guaiacol; 1000 mg/L for 4EP; 5 μg/L for IBMP; and 1 μg/L for TCA. Base wine (2.55 L) was then spiked with an appropriate volume of stock solution to achieve the concentrations of each volatile compound required for detection threshold determinations. For each compound, three sets of two control wines and one spiked was presented per judge. As there were 34 judges, 5.1 L of control wine was needed, and 2.55 L of spiked for each compound. Using a 100 mg/L stock solution of guaiacol, 230 μL were added to each liter of de-aromatized red wine to achieve a 23 μg/L concentration in the sample. The stock solution of *o*-cresol was also 100 mg/L, so 620 μL was used per liter of wine to achieve the 62 μg/L detection threshold level. 4EP was spiked with 0.605 mL of 1000 mg/L to reach 605 μg/L. The stock solution concentrations of IBMP and TCA were lower, and 3 mL and 4 mL of each was used to achieve a final concentration of 15 ng/L and 4 ng/L odor detection threshold levels for each compound, respectively. Base wine was spiked within 24 h of sensory analysis and stored at 5 °C in the dark. Stock solutions were stored at 5 °C in brown, sealed glass bottles, with the exception of the IBMP stock solution, which was stored at −20 °C in foil-wrapped containers to prevent light incursion.

2.3. Panel Selection

Thirty four participants (ages ranging from 21–54 years) were recruited from an existing pool of individuals who had previously volunteered for sensory evaluation testing in our facility, and were familiar with the format, task, and procedures involved in triangle testing. Panelists were screened against inclusion criteria comprising being of legal drinking age in South Africa and consuming wine at least once every six months. Exclusion criteria comprised being a smoker and having taste or smell defects. The panel consisted of four males and 30 females of varying abilities from novices to very experienced wine tasters. All participants provided their informed consent before participating in the study. All sensory data was obtained in compliance with institutional procedures for sensory evaluation (Ethical Clearance VIT-2018-6570). For the purposes of this study, "experienced" judges (n = 18) were those who had been members of wine sensory panels within the university and/or the wine industry for at least six months. "Inexperienced" judges (n = 16) were not members of sensory panels, and although they indicated they regularly consumed wine, most had no formal wine sensory training.

2.4. Sensory Testing

A triangle test was carried out to test the sensitivity of panelists for the selected compounds (three volatile phenols, TCA, and IBMP) in red wine. As this was not a full detection threshold determination, the investigation was carried out using a simplified version of the triangle test method [8].

Sensory evaluation of the wines was conducted in a sensory laboratory equipped with individual booths with standard artificial daylight lighting and temperature control at 20 ± 1 °C. One hour before serving, wines were equilibrated to room temperature before being poured into black ISO 3591:1977 standard glasses and covered with plastic lids. Each panelist worked in a white isolated booth and no communication was permitted between panelists. Testing for all five compounds was conducted on the same day, in triplicate. Participants therefore received five sets of 30 mL samples, one for each compound of interest. Each set contained brackets of two control (clean) wines, and one wine which had been spiked with one of five compounds under investigation. The samples were labelled with individual three digit codes and presented in a randomized order per panelist as recommended by Lawless and Heymann [2]. Panelists only evaluated samples orthonasally, as the study was concerned only with odor thresholds and aroma effects, and not with palate effects. From each set, panelists were asked to select the odd (or different) sample. Judges tested the compound ODT in the following order: Guaiacol, *o*-cresol, 4EP, IBMP, and TCA. To cancel strong carry-over effects and to minimize tiredness of the sense organs, judges were asked to rest for fifteen minutes between sets. Results of the triangle test were then examined to determine whether judges' experience made a difference to their abilities to correctly identify spiked samples. Panelists were deemed sufficiently sensitive if two out of three replicates were correct.

2.5. Data Analysis

In each set, if a judge had 0 or 1 out of three brackets correct, the overall result for the judge was considered a "0" or incorrect (not detected). If the judge had two or three correct brackets, the judge was considered a "1" or correct (spiked sample detected). Results were counted to determine whether 50% or more of the judges had detected samples spiked at the detection threshold. Counts were compared to the minimum number of correct judgments required for significance at probabilities of 5% and 1% for the triangle test (one-tailed) [26]. The data were separated into "experienced" ($n = 18$) and "inexperienced" judge ($n = 16$) groupings and any differences between groupings during the ODT investigated. This raw "judge response" data was further analyzed post hoc to investigate perceptual relationships between compounds and categorical factors using Excel 2017 (Microsoft Office, Redmond, WA, USA) and Statistica 12. The additional analyses were carried out using mixed model ANOVA. Least Squares Means (LSM) were generated for the panelists and per compound using this data. When a significant effect was observed, the Fisher's Least Significant Difference (LSD) test was used to compute pairwise comparisons with $\alpha = 0.05$. The quantitative factors were analyzed by Spearman's and Pearson's correlations.

3. Results

3.1. Investigation of Odor Detection Thresholds

To confirm an odor detection threshold using the standard sensory methodology, the number of judges selecting the different (spiked) samples correctly must be at or around 50% of the participants [8]. Roessler tables [44] indicate that if 34 judges are used to carry out the triangle test (as in this case), results are significantly different from the control at the 5% level ($p = 0.05$) if 17/34 judges make the correct choice over the complete set for a compound (i.e., 50% of the participants). If 19/34 judges choose correctly, the results are significant at the 1% level ($p = 0.01$). Results are presented in Figures 1–4. None of the compounds were therefore at exactly their ODTs for this matrix. As can be seen in Figure 1, *o*-cresol was most easily detected at its ODT, 4EP and guaiacol were also successfully detected by a significant number of judges, and TCA and IBMP were less easily detected in this matrix.

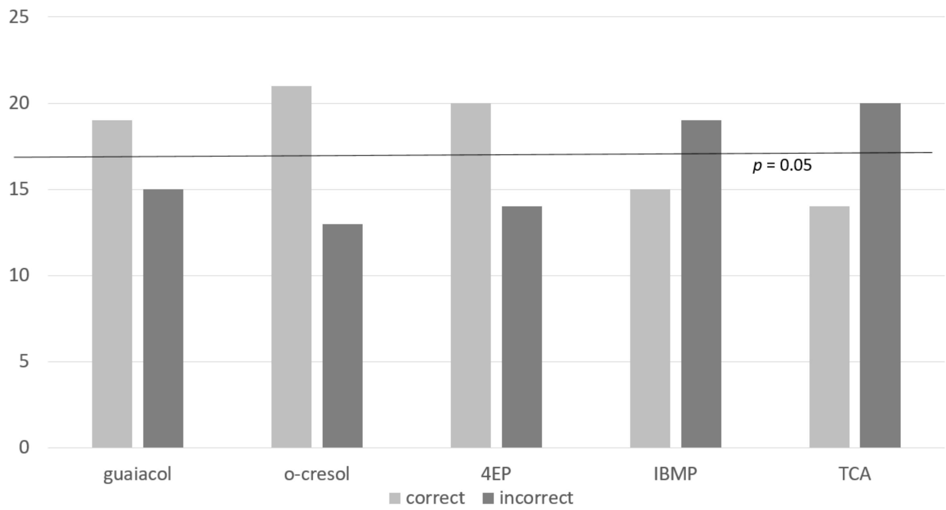


Figure 1. Correct vs incorrect judgments per compound (n = 34 judges).

In this study, the de-aromatized Shiraz wine was spiked with the equivalent of 23 µg/L of guaiacol. On a per judge (n = 34) basis, taking two or more responses as correct per triad, 19 judges chose correctly, and 15 chose incorrectly. This again is significant at p = 0.01 [44]. This indicated that guaiacol, spiked in de-aromatized Shiraz at a concentration of 23 µg/L, was detected by the majority of tasters and therefore the ODT was confirmed.

For the second volatile phenol in this study, 21 of the 34 judges were able to detect *o*-cresol at this level (62 µg/L) in de-aromatized Shiraz wine. This result is significant at p = 0.01 level. This means that a spiking level of 62 µg/L is higher than the ODT for *o*-cresol in this matrix. Polyphenols and ethanol have both been shown to have an effect on the perception of volatile phenols [37], and with other aroma compounds removed, the *o*-cresol may have been more obvious to the judges in this study than those involved in previous studies with fully aromatic red wine.

Very similar results to those of the other two volatile phenols were obtained for 4EP. Twenty (59%) of the 34 judges were able to detect a difference between samples spiked with threshold levels of 4EP in a de-aromatized Shiraz. This result was significant for the triangle test at p = 0.01.

In this study, the detection threshold of 15 ng/L for IBMP was chosen, because although Roujou de Boubée et al. [24] found higher levels in some French red wines, the wine in this study was unwooded and partially de-aromatized, and it was felt that a higher concentration might be too easily detected. Despite this, participants had difficulty detecting IBMP in the de-aromatized Shiraz matrix at this level. Only 15 (44%) of the 34 judges were able to detect a difference between samples which was not significant. Van Wyngaard et al. [45] showed that IBMP had an interactive effect on thiols in solution in Sauvignon blanc. Although there is not a lot of research on the masking effect of IBMP, other research [34] has shown, using canonical variate analysis, that wines spiked with either bell pepper or fruit were separated on the fruit/bell pepper continuum, i.e., that a masking effect of vegetative aromas by fruit aromas occurred. It may be the case in this study that the fruity aromas of the Shiraz masked the vegetative green pepper aroma for the less experienced judges, or that these judges felt sensory fatigue.

Participants also had difficulty detecting TCA in the de-aromatized Shiraz matrix. Only 14 judges (41%) of the 34 judges were able to detect a difference between controls and samples spiked with threshold levels of TCA at the 4 ng/L level. This finding substantiates others [9] that higher levels are needed in a red wine matrix as it is more complex. TCA is known to be a particularly "sticky" compound [40] with a potent inhibitory effect on olfactory cell responses, showing slow kinetics with an integration time of approximately 1 s. Even at infra-threshold levels, TCA has a marked masking effect on other odors [25], and these effects are sustained. It is also well established that judges vary substantially in their abilities to detect TCA: from 1 to 250 ng/L amongst experienced panelists, and from 2.5 ng/L to 25,000 ng/L in inexperienced judges [46]. As in the case of IBMP, it is possible that judges experienced sensory fatigue by this stage of the testing, and masking effects of other compounds may have decreased sensitivity of panelists to TCA. However, the ODTs of IBMP and TCA may be at higher concentrations in de-aromatized Shiraz, and an additional study, with increased concentrations of both compounds, would be useful to confirm this. The judge responses in detection of volatile phenols, and the latter two compounds show that although a single level provides some information regarding the ODT in a specific matrix, to truly establish the ODT for a compound, it is necessary to carry out a full sensory study as recommended by ASTM 679-04 [8].

3.2. Differences in Frequency of Correct Detection between Compounds by Panelists

Table 2 shows the results for Pearson's (linear relationship) and Spearman correlation (monotonic: Whether linear or not) coefficients between compounds in solution, with −1.0 indicating a perfect negative correlation, and 1.0 indicating a perfect positive correlation. Values for relationships varied between −0.13 and 0.27, indicating there are only very weak positive and negative correlations between some of the variables. This is substantiated by the *p* values, none of which are below 0.11, an indication that relationships that do exist are not significant. The results of the two analyses therefore gave the same outcomes.

Table 2. Correlation strength between compounds according to Pearson and Spearman correlation coefficients of correct identification of spiked samples by tasters between compound pairs.

Triangle Test Correlations between Compounds (n = 34)					
Compound 1	**Compound 2**	**Pearson**	**Pearson p**	**Spearman**	**Spearman p**
o-Cresol	4EP	−0.06	0.74	−0.05	0.78
o-Cresol	Guaiacol	−0.13	0.46	−0.10	0.56
o-Cresol	IBMP	0.19	0.28	0.19	0.27
o-Cresol	TCA	0.06	0.75	0.04	0.82
4EP	Guaiacol	0.08	0.65	0.06	0.74
4EP	IBMP	0.00	0.98	0.03	0.88
4EP	TCA	0.28	0.11	0.27	0.12
Guaiacol	IBMP	0.24	0.17	0.24	0.17
Guaiacol	TCA	0.16	0.37	0.14	0.42
IBMP	TCA	0.01	0.96	0.02	0.91

In a second attempt to identify any relationships between compounds, the number of correct selections within the three repeats were added, giving a score between 0–3, and this was analyzed in the standard way using mixed model ANOVA (Table 3). A generalized estimating equations (GEE) analysis on the raw 0/1 outcomes was carried out. The GEE method is often used to analyze longitudinal and other correlated response data, especially if responses are binary [47], as in this comparison.

Table 3. Least Significant Difference comparisons between compound pairs.

Comparisons	**1st Mean**	**2nd Mean**	**Mean Diff**	p
1-2	Guaiacol	*o*-Cresol	−0.41	0.09
1-3	Guaiacol	4EP	−0.15	0.54
1-4	Guaiacol	IBMP	0.18	0.47
1-5	Guaiacol	TCA	0.35	0.15
2-3	*o*-Cresol	4EP	0.26	0.28
2-4	*o*-Cresol	IBMP	0.59	0.02
2-5	*o*-Cresol	TCA	0.76	0.00
3-4	4EP	IBMP	0.32	0.18
3-5	4EP	TCA	0.50	0.04
4-5	IBMP	TCA	0.18	0.47

In this case, most pairs of compounds showed no significant differences in detection by panelists, but there were two exceptions. Judges who detected *o*-cresol seemed to have been able to detect IBMP and TCA more readily and there was also a weak tendency for judges who detected 4EP to be able to detect TCA more readily. This would need to be tested with a more directed study looking at different levels of compounds before any assumptions about these relationships could be made.

For the purposes of further exploring relationships and panelists' responses, compounds were grouped into aroma families according to Noble et al. [48]. The volatile phenols (guaiacol, 4EP, and *o*-cresol) encompassed a group of descriptors that fell into the "woody" category, and IBMP into "vegetative" and TCA into "earthy". There did appear to be significant differences in panelists' abilities to detect between odor families. There was a definite predominance (21 of the 34 judges, or 62%) of correct choices associated with the phenolic "woody" family of compounds across both groups (experienced as well as inexperienced panelists). The TCA/IBMP group predominated in correct identifications in only eight (23%) of the judges, and in five (15%) judges' cases, there was no predominance of either aroma family.

The hedonic valence of an odor has been shown to influence its perception by panelists [41]. The predominance of correct identification associated with volatile phenols may be because humans generally associate woody, burnt smells with cooking/food and home fires, and therefore prefer and detect them. It could also be that judges unconsciously avoided selecting samples with moldy or unripe (green) characters, as the ability to be put off unsuitable/unhealthy food before tasting or ingesting it may confer an evolutionary advantage through infectious disease [49]. Another explanation may be that panelists have different sensitivities for different groupings of compounds based on their cultural backgrounds, as found by previous works [39,40]. These suppositions in the context of this study need to be tested with further research.

3.3. The Impact of Previous Training or Exposure on Judge Sensitivity

Tempere et al. [50] noted that even wine tasting "experts" show high olfactory detection thresholds for key compounds of wine, which is not ideal when wine quality depends on fault-detection at low levels. Kaeppler and Mueller [41] raised the question whether olfactory systems may be in fact linguistic arrangements based on experience and exposure to expert lexicons. Whether odor categories are innate or learned depends on the influence of language on odor processing. To investigate whether previous training or exposure of a participant (for example, as a member of a sensory panel) impacted sensitivity to threshold differences between samples and controls, panelists in this study were separated into "experienced" and "inexperienced" groups, and differences in their responses/results during the sensitivity study were investigated.

Figure 2 shows the results for individual judges, and their abilities to detect differences between spiked and control samples. Out of 34 panelists in our trial, five did not detect guaiacol at all, four could not detect *o*-cresol, and six judges of the 34 (not the same people) were unable to detect 4EP, TCA, and IBMP. Out of the 34, there were 15 judges who detected all the compounds, and two judges who were unable to correctly identify any of the spiked samples. The average number of compounds correctly identified by experienced judges was 3.69, with a median of 4, and an SD of 0.7. Inexperienced judges, on the other hand, had an average for correct judgments of only 1.72, with a median of 2.00 (SD 0.89). In the inexperienced group, none of the judges were able to perceive all five compounds in spiked solutions. It is obvious, therefore, that training and experience must have sensitized the experienced judges to the very subtle differences between wines.

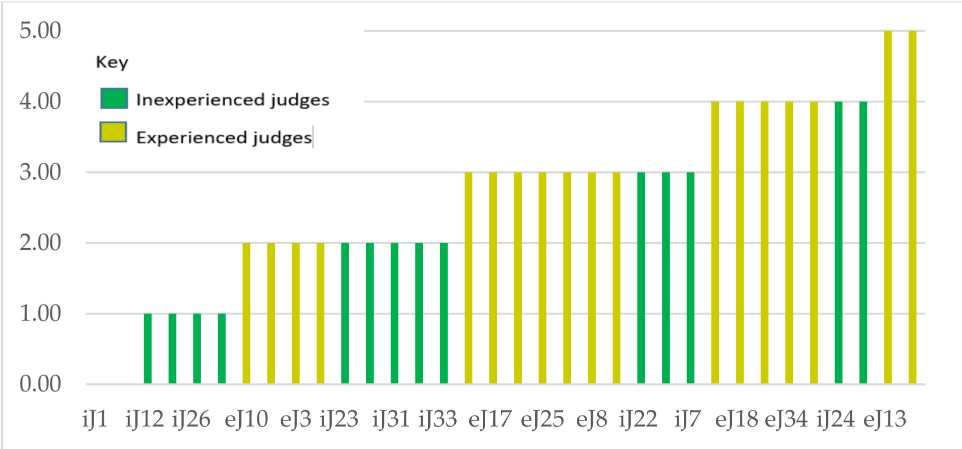


Figure 2. Number of correct judgements per experienced (ej) and inexperienced judge (ij) by 3-AFC Triangle Test for five compounds.

The Least Squares Means (LSM), generated using the mixed model ANOVAs for inexperienced vs. experienced judges (p = 0.04) indicate that the overall group effect is significant (Figure 3); experienced judges were better able to correctly identify spike samples from controls. This could be due to higher smell acuity generally or through experience working on wine evaluation panels. In other circumstances (for example, research project panels that require a particular acuity or sensitivity for a specific compound), it may have been advisable to exclude judges with poor or inconsistent performance, and/or omitting their observations from the data set. This concurs with what other researchers have recommended [14], that adapted training for professionals in the wine industry will enhance abilities of judges to differentiate between spiked samples and controls, even at ODT levels.

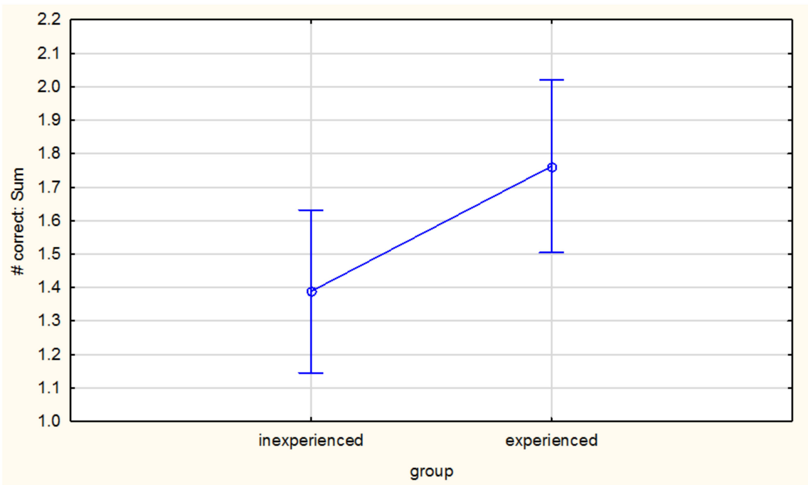


Figure 3. Group effect (Least Squares Means) of experienced vs inexperienced judges (Vertical bars denote 0.95 confidence intervals).

Figure 4 indicates averages of judgements for each compound (out of three) across all the judges. Only trends can be seen, for example, experienced judges seemed better able to detect the spiked samples in the case of guaiacol, *o*-cresol, and IBMP, but these differences were not significant (p = 0.38). There was no difference at all between judges, whether experienced or inexperienced in detecting the spiked TCA samples, and little difference in the case of 4EP. As IBMP and TCA are the two compounds that appear to show the lowest number of correct judgments, it is quite probable that the assessors also experienced sensory fatigue. Ideally, these compounds should have been tested on different days.

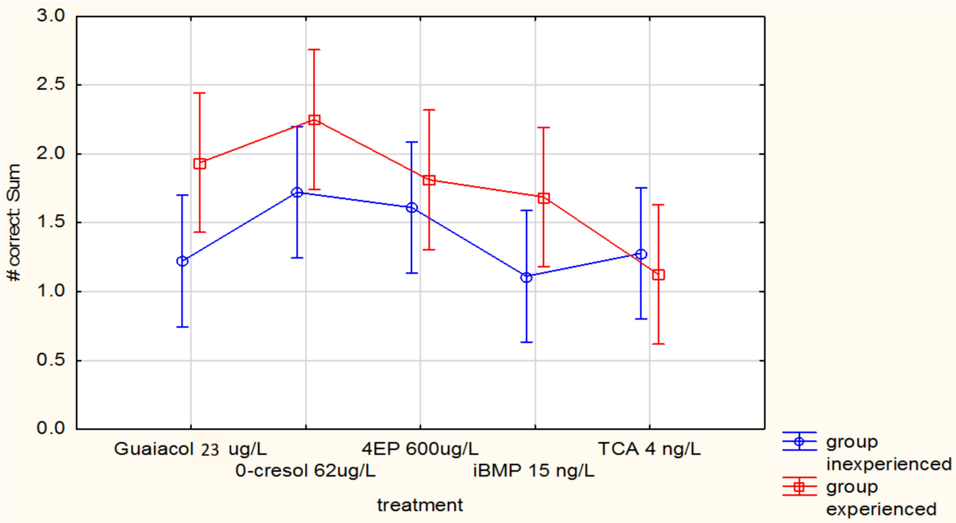


Figure 4. Number of correct judgements per experienced and inexperienced judge by 3-AFC Triangle Test (LS means) for individual compounds. Vertical bars denote 0.95 confidence intervals.

4. Conclusions

To explore a pragmatic approach to judge sensitivity for five taint compounds (guaiacol, *o*-cresol, 4EP, TCA, and IBMP) in red wine, three triangle tests (two controls, one sample spiked at the selected ODT for each compound) were carried out for 34 judges. Spiked samples were correctly differentiated from controls in the following order: *o*-cresol (62% correct), 4EP (59% correct), guaiacol (56% correct), TCA (44% correct), and IBMP (41% correct). What could be deduced from an analysis of correct identification of spiked compounds was that correlations in perception between pairs of compounds was very weak. If a particular compound was detected by the judges, it did not have a bearing on another group, but judges that had higher scores generally were more likely to detect all compounds across both families. As IBMP and TCA were the last two compounds to be assessed of the five, and also the two compounds that showed the least correct responses or differences between experienced and inexperienced judges, it is probable that the assessors experienced sensory fatigue. This is a limitation of this strategy, and if the study were to be repeated, it would be advisable for each compound to be tested on a different day at different levels, or presented in a random order to the judges. Ideally, a full ODT study should be carried out to determine ODTs for a specific matrix, but the simplified method will be a frame of reference for further work. In this study, the responses of the judges confirmed that the ODTs for guaiacol, and 4-ethylphenol in de-aromatized Shiraz wine, but an additional, more comprehensive sensory study to establish whether the levels could be reduced would be useful to determine the actual thresholds of detection in this matrix.

The concentration of *o*-cresol could certainly be reduced in future work in this matrix, as almost two thirds of the panelists were able to detect spiked samples, and concentrations of IBMP and TCA could be increased, as only 44% and 41% of judges could distinguish these compounds spiked at accepted ODT levels in this matrix. This may also have been due to sensory fatigue, and is a limitation of testing this number of compounds on one day.

One interesting aspect that emerged from this work was the ability of judges to detect compounds more easily if they had some experience of winetasting, showing that training can possibly sensitize people to attributes that they might not have noticed previously. The confirmation of ODTs in a partially de-aromatized red wine matrix has opened the way for further research and the possibility of training industry members to sensitize them to these off-flavors. The unique and complex interactions between language, training, and olfaction should definitely be assessed in future studies. This study highlights how important it is to understand the detection thresholds of compounds in different products, and has relevance for many foods and beverages where sensory thresholds from the literature may be taken for granted and not tested in the study matrix.

Author Contributions: M.M., A.B. and F.F.B. conceived and designed this study; V.P. carried out the detection threshold practical work; M.M. wrote the paper, which was revised and edited by A.B., F.B. and V.P.

Funding: This research received no external funding.

Acknowledgments: Winetech and THRIP (NRF) acknowledged funding associated with volatile phenol projects. Lucky Mokwena of the Central Analytical Facility, Stellenbosch University, performed volatile phenol analyses. Martin Kidd carried out the statistical analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Perry, D.M.; Hayes, J.E. Effects of matrix composition on detection threshold estimates for methyl anthranilate and 2-Aminoacetophenone. *Foods* **2016**, *5*, 35. [CrossRef] [PubMed]
2. Lawless, H.T.; Heymann, H. *Sensory Evaluation of Foods: Principles & Practices*, 2nd ed.; Springer Science Business Media LLC: New York, NY, USA, 2010.
3. American Society for Testing and Materials ASTM E1432-04 (2011). Standard Practice for Defining and Calculating Individual and Group Sensory Thresholds from Forced-Choice Data Sets of Intermediate Size. Available online: https://www.astm.org/Standards/E1432.htm (accessed on 25 August 2018).
4. Martineau, B.; Acree, T.E.; Henick-Kling, T. Effect of wine type on the detection threshold for diacetyl. *Food Res. Int.* **1995**, *28*, 139–143. [CrossRef]
5. Brown, K.; Maclean, C.M.; Robinette, R.R. The distribution of the sensitivity to chemical odors in man. *Hum. Biol.* **1968**, *40*, 456–472. [CrossRef] [PubMed]
6. Pickering, G.J.; Karthik, A.; Inglis, D.; Sears, M.; Ker, K. Determination of Ortho- and Retronasal Detection Thresholds for 2-Isopropyl-3-Methoxypyrazine in Wine. *J. Food Sci.* **2007**, *72*, S468–S472. [CrossRef] [PubMed]
7. Wood, C.; Siebert, T.E.; Parker, M.; Capone, D.L.; Elsey, G.M.; Pollnitz, A.P.; Eggers, M.; Meier, M.; Vössing, T.; Widder, S.; et al. From wine to pepper: Rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *J. Agric. Food Chem.* **2008**, *56*, 3738–3744. [CrossRef] [PubMed]
8. American Society for Testing and Materials ASTM E679-04(2011). Standard Practice for Determination of Odor and Taste Thresholds by a Forced-Choice Ascending Concentration Series Method of Limits. Available online: https://www.astm.org/Standards/E679.htm (accessed on 10 May 2018).
9. Mazzoleni, V.; Maggi, L. Effect of wine style on the perception of 2,4,6-trichloroanisole, a compound related to cork taint in wine. *Food Res. Int.* **2007**, *40*, 694–699. [CrossRef]
10. Yang, W.; Li, W.; Liu, B. Odour prediction model using odour activity value from pharmaceutical industry. *Austrian Contrib. Vet. Epidemiol.* **2015**, *8*, 51–60.
11. Villamor, R.R.; Ross, C.F. Wine matrix compounds affect perception of wine aromas. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 1–20. [CrossRef] [PubMed]
12. Lorrain, B.; Tempere, S.; Iturmendi, N.; Moine, V.; De Revel, G.; Teissedre, P.-L. Influence of phenolic compounds on the sensorial perception and volatility of red wine esters in model solution: An insight at the molecular level. *Food Chem.* **2013**, *140*, 76–82. [CrossRef] [PubMed]
13. Lapalus, E. Linking Sensory Attributes to Selected Aroma Compounds in South African Cabernet Sauvignon Wines. Master's Thesis, Stellenbosch University, Western Cape, South Africa, March 2016.
14. Tempere, S.; Cuzange, E.; Malak, J.; Bougeant, J.C.; De Revel, G.; Sicard, G. The training level of experts influences their detection thresholds for key wine compounds. *Chemosens. Percept.* **2011**, *4*, 99–115. [CrossRef]
15. Larcher, R.; Nicolini, G.; Bertoldi, D.; Nardin, T. Determination of 4-ethylcatechol in wine by high-performance liquid chromatography–coulometric electrochemical array detection. *Anal. Chim. Acta* **2008**, *609*, 235–240. [CrossRef] [PubMed]
16. Curtin, C.; Bramley, B.; Cowey, G.; Holdstock, M.; Lattey, K.; Coulter, A.; Henschke, P.; Francis, L.; Godden, P. Sensory perception of Brett and relationship to consumer preference. In Proceedings of the 13th Australian Wine Industry Technical Conference, Adelaide, Australia, 27 July–2 August 2007; pp. 207–211.
17. Bearak, B. A Whiff of Controversy and South African Wines—The New York Times. Available online: https://www.nytimes.com/2009/06/29/world/africa/29stellenbosch.html (accessed on 26 June 2018).

18. Botha, J.J. Sensory, Chemical and Consumer Analysis of Brettanomyces Spoilage in South African Wines. Master's Thesis, Stellenbosch University, Western Cape, South Africa, March 2010.
19. Hammond, C.E. South African Wine under Fire. Available online: http://www.carolynevanshammond.com/blog/2015/10/12/south-african-wine-under-fire-1 (accessed on 26 June 2018).
20. Van Eeden, P.R. Chemical, Sensory and Consumer Analysis of Cork Taint in South African Wines. Master's Thesis, Stellenbosch University, Western Cape, South Africa, March 2009.
21. Parker, M.; Osidacz, P.; Baldock, G.A.; Hayasaka, Y.; Black, C.A.; Pardon, K.H.; Jeffery, D.W.; Geue, J.P.; Herderich, M.J.; Francis, I.L. Contribution of several volatile phenols and their glycoconjugates to smoke-related sensory properties of red wine. *J. Agric. Food Chem.* **2012**, *60*, 2629–2637. [CrossRef] [PubMed]
22. Parker, B.M.; Baldock, G.; Hayasaka, Y.; Mayr, C.; Williamson, P.; Francis, I.L.; Krstic, M.; Herderich, M.; Johnson, D. Seeing through smoke. *Wine Vitic. J.* **2013**, *28*, 42–46.
23. Chatonnet, P.; Dubourdie, D.; Boidron, J.-N.; Pons, M. The origin of ethylphenols in wines. *J. Sci. Food Agric.* **1992**, *60*, 165–178. [CrossRef]
24. Roujou de Boubée, D.; Van Leeuwen, C.; Dubourdieu, D. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48*, 4830–4834. [CrossRef] [PubMed]
25. Prescott, J.; Norris, L.; Kunst, M.; Kim, S. Estimating a "consumer rejection threshold" for cork taint in white wine. *Food Qual. Prefer.* **2005**, *16*, 345–349. [CrossRef]
26. Boidron, J.N.; Chatonnet, P.; Pons, M. Influence du bois sur certaines substances odorantes des vins. *Connaiss. La Vigne Du Vin* **1988**, *22*, 275–294. [CrossRef]
27. Kennison, K.R.; Wilkinson, K.L.; Williams, H.G.; Smith, J.H.; Gibberd, M.R. Smoke-derived taint in wine: Effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. *J. Agric. Food Chem.* **2007**, *55*, 10897–10901. [CrossRef] [PubMed]
28. Spillman, P.J.; Iland, P.G.; Sefton, M.A. Accumulation of volatile oak compounds in a model wine stored in American and Limousin oak barrels. *Aust. J. Grape Wine Res.* **1998**, *4*, 67–73. [CrossRef]
29. Escudero, A.; Campo, E.; Fariña, L.; Cacho, J.; Ferreira, V. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J. Agric. Food Chem.* **2007**. [CrossRef] [PubMed]
30. Allen, M.S.; Lacey, M.J.; Boyd, S.J. Existence of different origins for Methoxypyrazines of grapes and wines. In *Biotechnology for Improved Foods and Flavors*; Takeoka, G.R., Teranishi, R., Williams, P.J., Kobayashi, A., Eds.; American Chemical Society: Washington, DC, USA, 1996; pp. 220–227.
31. PubChem. 2-Isobutyl-3-methoxypyrazine | C9H14N2O—PubChem. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/2-isobutyl-3-methoxypyrazine#section=Top (accessed on 19 June 2018).
32. Shibamoto, T. Odor threshold of some pyrazines. *J. Food Sci.* **1986**, *51*, 1098–1099. [CrossRef]
33. Tempere, S.; Schaaper, M.H.; Cuzange, E.; De Revel, G.; Sicard, G. Masking of several olfactory notes by infra-threshold concentrations of 2,4,6-trichloroanisole. *Chemosens. Percept.* **2017**, *10*, 69–80. [CrossRef]
34. Hein, K.; Ebeler, S.; Heymann, H. Perception of fruity and vegetative aromas in red wine. *J. Sens. Stud.* **2009**, *24*, 441–455. [CrossRef]
35. Cravero, M.C.; Bonello, F.; Del Pazo Alvarez, M.C.; Tsolakis, C.; Borsa, D. The sensory evaluation of 2,4,6-trichloroanisole in wines. *J. Inst. Brew.* **2015**, *121*, 411–417. [CrossRef]
36. Campo, E.; Ferreira, V.; Escudero, A.; Cacho, J. Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography−olfactometry data. *J. Agric. Food Chem.* **2005**, *53*, 5682–5690. [CrossRef] [PubMed]
37. Petrozziello, M.; Asproudi, A.; Guaita, M.; Borsa, D.; Motta, S.; Panero, L.; Bosso, A. Influence of the matrix composition on the volatility and sensory perception of 4-ethylphenol and 4-ethylguaiacol in model wine solutions. *Food Chem.* **2014**, *149*, 197–202. [CrossRef] [PubMed]
38. Kim, D. Understanding the Effects of Wine Matrix Compounds on the Perception of Aromatic Wine Faults. Ph.D. Thesis, University of Guelph, Guelph, ON, Canada, December 2016.
39. Chrea, C.; Valentin, D.; Sulmont-Ross, C.; Ly Mai, H.; Hoang Nguyen, D.; Abdi, H. Culture and odor categorization: Agreement between cultures depends upon the odors. *Food Qual. Prefer.* **2004**, *15*, 669–679. [CrossRef]
40. Takeuchi, H.; Kato, H.; Kurahashi, T. 2,4,6-Trichloroanisole is a potent suppressor of olfactory signal transduction. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 16235–16240. [CrossRef] [PubMed]

41. Kaeppler, K.; Mueller, F. Odor classification: A review of factors influencing perception—Based odor arrangements. *Chem. Sens.* **2013**, *38*, 189–209. [CrossRef] [PubMed]
42. Wilson, C.L. Chemical Evaluation and Sensory Relevance of Thiols in South African Chenin Blanc Wines. Master's Thesis, Stellenbosch University, Western Cape, South Africa, March 2017.
43. De Vries, C.J.; Mokwena, L.M.; Buica, A.; McKay, M. Determination of volatile phenol in Cabernet Sauvignon wines made from smoke-affected grapes, by using HS-SPME GC-MS. *S. Afr. J. Enol. Vitic.* **2016**, *37*, 15–21. [CrossRef]
44. Roessler, E.B.; Pangborn, R.M.; Sidel, J.L.; Stone, H. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. *J. Food Sci.* **1978**, *43*, 940–943. [CrossRef]
45. Van Wyngaard, E.; Brand, J.; Jacobson, D.; Du Toit, W.J. Sensory interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatised Sauvignon Blanc wine. *Aust. J. Grape Wine Res.* **2014**, *20*, 178–185. [CrossRef]
46. Sefton, M.A.; Simpson, R.F. Compounds causing cork taint and the factors affecting their transfer from natural cork closures to wine—A review. *Aust. J. Grape Wine Res.* **2005**, *11*, 226–240. [CrossRef]
47. Hanley, J.A.; Negassa, A.; DeB Edwardes, M.D.; Forrester, J.E. Statistical analysis of correlated data using generalized estimating equations: An orientation. *Am. J. Epidemiol.* **2003**, *157*, 364–375. [CrossRef] [PubMed]
48. Noble, A.C.; Arnold, R.A.; Buechsenstein, J.; Leach, E.J.; Schmidt, J.O.; Stern, P.M. Modification of a standardized system of wine aroma terminology. *Am. J. Enol. Vitic.* **1987**, *38*, 143–146.
49. Curtis, V.; De Barra, M.; Aunger, R. Disgust as an adaptive system for disease avoidance behaviour. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2011**, *366*, 389–401. [CrossRef] [PubMed]
50. Tempere, S.; Cuzange, E.; Bougeant, J.C.; De Revel, G.; Sicard, G. Explicit sensory training improves the olfactory sensitivity of wine experts. *Chemosens. Percept.* **2012**, *5*, 205–213. [CrossRef]

Chapter 5

Perceptual interactions and characterisation of odour quality of binary mixtures of volatile phenols and IBMP in a red wine matrix

This article is written in the style of the South African Journal of Enology and Viticulture for submission

Perceptual interactions and characterisation of odour quality of binary mixtures of volatile phenols and 2-isobutyl-3-methoxypyrazine in a red wine matrix

Marianne McKay [1*], Florian F. Bauer [2], Valeria Panzeri [3] and Astrid Buica[4]

[1]Department of Viticulture and Oenology; Stellenbosch University, marianne@sun.ac.za

[2]Department of Viticulture and Oenology; Stellenbosch University, fb2@sun.ac.za

[3]Institute for Grape and Wine Sciences; Stellenbosch University panzeri@sun.ac.za

[4]Department of Viticulture and Oenology; Stellenbosch University abuica@sun.ac.za

Keywords: binary mixtures; interactions; volatile phenols; sensory effects; IBMP; DA;

Running title: *Sensory effects of binary mixtures of volatile phenols and IBMP in red Wines*

Abstract

The qualitative perceptual interactions of single and binary mixtures of four compounds: guaiacol (burnt/smoky), *o*-cresol (phenolic/tar), 4-ethylphenol (4-EP, leather/barnyard), and 2-isobutyl-3-methoxypyrazine (IBMP, green pepper/herbaceous), were studied in a partially de-aromatised red wine matrix. Using Descriptive Analysis, each compound was characterised individually by a trained panel of eleven judges at peri- and sub-threshold levels. Binary mixtures of all four compounds were subsequently characterised in the same matrix using a D-optimal fractional statistical design. The data show that the olfactory outcome of binary mixtures in red wine cannot be predicted from the attributes of single compounds, with some 'single compound' attributes absent in binary mixtures, and new, attributes emerging when two compounds are present together. This work adds to our understanding of the sensory impact of low levels of volatile phenols and IBMP in wine.

1. Introduction

Problematic compounds, including volatile phenols, may accrue during the production process and lead to off-flavours and/or lack of typicality of wine style. These issues are often detected by olfaction (Perry & Hayes, 2016). Frequently used indicators of the potency of aroma compounds associated with aroma in wine and many other matrices include odour detection threshold (ODT) and odour activity value (OAV) measurements. Odour thresholds are ill-defined in theory (Lawless & Heymann, 2010), but are based on the lowest concentration of a compound at which individuals can perceive a difference in aroma quality, relative to a control. The OAV is defined as the concentration of a compound present in a matrix divided by the ODT for that compound in that specific matrix. Generally, the larger the OAV, the more likely that compound would contribute to the overall odour of a complex odour mixture (Yang *et al.*, 2015). In theory, ODTs and OAVs may be determined this way for compounds of interest (Yang *et al.*, 2015) but in practice, a large number of issues influence olfactory perception, including matrix effects and the so-called ‘sensory interaction’ of compounds which affects their perception by judges.

It is well-known that the process of olfaction begins when volatile molecules enter the nostrils and activate receptors in the olfactory epithelium. Olfactory receptor proteins (ORP) are located in hair-like projections of the olfactory sensory neurons (cilia), and their activation produces response signals which are detected by the olfactory bulb (OB), and travel from the OB to the piriform cortex (PC) (Wilson & Stevenson, 2010). Output from the PC goes to various other brain regions and, combined with other inputs, is interpreted as a perception of odour in the orbitofrontal cortex (OFC) (Sell, 2006). The connections between olfactory organs, the emotional system (limbic system) and long-term memory of the human brain are complex, and have not been fully elucidated. The main features of monomolecular (single odour) olfactory processing are, however, well- characterised: Odour intensity is driven primarily by odorant concentration, and odour quality is related to molecular structure (Sell, 2006), odour intensity (Wilson & Stevenson, 2010) and individual cognitive factors (Burdach *et al.*, 1985).

Perception of odour mixtures, such as those present in wine, is far more complicated due to interactions arising from the complex chemical signal encoding and processing within the olfactory system (Thomas- Danguin *et al.*, 2014). The theory of ‘odour-object’ encoding underpins the neurophysiological processes involved in extracting only relevant information from complex chemical mixtures in the environment (Wilson& Stevenson, 2010; Thomas-Danguin *et al.*, 2014). The overlapping response profiles of olfactory sensor neurons (OSNs) and subsequent encoding is under cognitive control, and continued exposure will lead to the experience of mixtures as odour-objects or specific odour configurations (Thomas-Danguin *et al.*, 2014). The perceptual configuration of mixtures into simpler odour-objects would thus improve an organism’s ability to extract information from the environment (Wilson & Stevenson, 2010), and cognitive processes should decrease the chemical complexity of the environment by building experience-dependent perceptual associations (Wilson & Stevenson, 2010).

Studies in different species have compared the responses of OSNs to binary mixtures and their components (Thomas-Danguin *et al.*, 2014), and data modelling (Munch *et al.*, 2013) suggests that both competitive and non-competitive interactions occur at receptor level. Thomas-Danguin *et al.*, (2014) note that there is competitive interaction when two molecules bind to the same receptor binding site which might involve agonist (molecules that activate the receptor) odorants, or agonist / antagonist (a molecule that binds to the receptor but is unable to activate it) competition. Chaput *et* *al.*, (2012), for example, showed competitive interaction in wine between whiskey lactone and isoamyl acetate, with the perception of ‘fruity notes’ of a mixture increased by low concentrations of whiskey lactone and decreased by high concentrations. The presence of other compounds in solution (so-called matrix effects) can also change the perception of target aromas, not just above the ODT (supra-threshold), but also below it (i.e. at sub- or infra-threshold). When the impact of infra- and supra-threshold concentrations of ethylphenols on wine was investigated by Tempere *et al.*, (2016), it was found that both sub- and suprathreshold concentrations of off-flavours not only changed the “hedonic valence” (positive or negative character) of the perception, but had a masking effect on fruity notes. The response intensity of OSNs to a mixture can also be higher than that induced by the most powerful component (partial addition) or exceed the summed response of all the components (hyper-addition). In most cases, a given type of interaction was sustained over a range of concentrations, but in some cases, a shift to another interaction type as a function of odorant concentration was reported (Rospars *et al.*, 2008).

Rationale for this study: In wine, volatile phenols (VPs) are not uncommon and can be derived from storage in oak barrels which have undergone toasting/firing during production (Chatonnet *et al.*, 1992), but elevated levels are associated with *Brettanomyces* infections (Romano *et al.*, 2009), oak aging of wine (Chatonnet, *et al.*, 1992) and smoke taint (Kennison, 2013). Although the attributes and odour detection thresholds of a number of VPs are discussed in the literature. (Czerny *et al.*, 2011), the characteristics of specific VPs in combination with each other or other compounds are largely unexplored. It has been shown that acetic acid and ethylphenols show olfactory antagonistic effects and mask fruity notes in a wine (Atanasova, Thomas-Danguin, Langlois, *et al.*, 2005) but the effects of individual compounds and their binary mixtures in wine has not been addressed. In this study, four odour- active compounds were selected that had been associated with specific off-flavour issues in wine aroma (Table 1), namely guaiacol, *ortho*-cresol (*o*-cresol), 4-ethylphenol (4-EP4-EP), 3-isobutyl-and 2-methoxypyrazine (IBMP). Aroma descriptors for these compounds cover a continuum from ‘burnt, smoky’ and ‘medicinal’ to the ‘herbaceous, green’ aromas associated with IBMP.

Table 1. Odour Detection Thresholds (ODTs) in µg/L (unless otherwise indicated) in red wine and descriptors for compounds used in this study

Compound	ODT (µg/L)	Descriptors	Reference
guaiacol	23 (red)	burnt, smoky, toasty, phenolic	(Parker *et al.*, 2012)
o-cresol	62 (red)	burnt, smoky, medicinal, tar	(Parker *et al.*, 2013)

4-EP	605 (red)	leather, bacon, medicinal, horse	(Chatonnet *et al.*, 1992)
IBMP	15 ng/L (red)	green, herbaceous, bell pepper'	(Roujou de Boubée *et al.*, 2000)

The aim of this study was to investigate the sensory effects of guaiacol, *o*-cresol, 4-ethylphenol, and 2- methoxy-3-isobutylpyrazine (IBMP) in red wine when present separately or in paired combinations. These four compounds were selected for investigation as they had been linked to the attributes associated with red wines which had been variously described as 'burnt rubber', 'dirty' (Bearak, 2009) and herbaceous/green' (Heyns, 2014). This study follows the confirmation of odour detection thresholds for various compounds in red wine, and testing of panel sensitivities, and serves as an exploratory step for the sensory profiling of these compounds in various combinations. This should prove useful for winemakers after smoke exposure of grapes, or when considering IBMP levels associated with the different ripening stages of red grapes, as interactions between compounds could possibly determine attributes in the final wine.

2. Materials and Methods

2.1 Base wine

A 2016 Shiraz wine (300 L) was supplied by a local wine producer (Koelenhof Cellar Ltd, Simonsberg, South Africa) and stored at 4˚C in 25 L food-grade plastic containers under nitrogen at the Department of Viticulture and Oenology, Stellenbosch University, South Africa. The wine had a pH of 3.6, and an alcohol concentration of 13% v/v as determined by the supplier, who confirmed that the wine had not been treated with wood at any time during the winemaking process. Informal benchtop screening by five experienced sensory judges with tested sensitivity for the aroma compounds used to spike the wines confirmed that those compounds were not present in the base wine, which had an odour profile that was dominated strongly by fruit. This warranted partial de-aromatization by mixing thoroughly with activated charcoal powder (Merck, Darmstadt, Germany) for 12 hours following the method outlined by Wilson, et al., (2017) prior to spiking for the interaction study. During the treatment and subsequent filtration, blending and storage steps, the wine was protected from oxidation under nitrogen gas. In a screening session, the expert panel chose a blend of 50:50 charcoal-treated wine to untreated wine which yielded a neutral wine base with low aromatic intensity. Samples of the base wine (50mL) were taken in order to determine the baseline levels of the compounds investigated in this study. Analysis of volatile phenols in the partially de-aromatised base wine was performed following the method outlined by (De Vries *et al.*, 2016) using an Agilent Gas Chromatograph, model 6890N (Agilent, Palo Alto, USA), coupled to an Agilent Mass

Spectrometer 5975 B Inert XL EI/CI (Agilent, Palo Alto, USA). The guaiacol level in the base wine was 1.37 µg/L, *o*-cresol was 0.08 µg/L and 4-ethylphenol concentration was 1.4 µg/L. The wine was also deemed, during informal tasting by the experienced sensory judges, to be completely free of any form of 'mouldy' or 'herbaceous' odours that might have been associated with IBMP.

2.2 Preparation of spiked wine samples

Stock solutions of 1000 mg/L of the four compounds were prepared in 99.5% ethanol (Merck Chemicals, South Africa). Guaiacol (99.3% purity), 4-EP (99.5% purity), *o*-cresol (99%), and IBMP (99%) were obtained from Sigma-Aldrich, South Africa. The compounds were dissolved in ethanol (10 mL) and then made up to volume with ultra-pure distilled water (Millipore, Bedford, MA, USA) to the concentrations required for spiking, i.e.: 100 mg/L for *o*-cresol and guaiacol; 1000 mg/L for 4-EP; and 5 µg/L for IBMP. Base wine was then spiked with an appropriate volume of stock solution to achieve the concentrations (Table 2) of each volatile compound required for detection threshold determinations. These solutions were used to produce wine samples spiked with the desired concentrations of each compound.

2.2.1 Preparation of 'singles': individual compounds at and below ODT

Using a 100 mg/L stock solution of guaiacol, 230 µL were added to each litre of de-aromatised red wine to achieve a 23 µg/L concentration in the sample. The stock solution of *o*-cresol was also 100 mg/L, so 620 µL was used per liter of wine to achieve the 62 µg/L detection threshold level. 4-EP was spiked with 0.605 mL of 1000 mg/L to reach 605 µg/L. The stock solution concentrations of IBMP was lower at 5 mg/L. All levels used were subjected to benchtop sensory pre-screening by five expert wine tasters/oenology researchers in order to determine whether they adhered to the sensory criteria set. For the subthreshold levels, the wine sensory experts established that 60-70% of the ODT for each volatile phenol compound did not cause easily identifiable changes from the aroma profile of the base wine. Base wine was spiked within 24 hours of sensory analysis and stored at 5˚C in the dark. Stock solutions were stored at 5˚C in brown, sealed glass bottles, with the exception of the IBMP stock solution which was stored at -20˚C in foil-wrapped containers to prevent light incursion. During the pre-screening, it was found that the 15 ng/L level of IBMP gave very strong 'green/herbaceous' attributes to the de-aromatised wine matrix, and lower levels were thus tested. It was decided that a level of 10 ng/L was just detectable as a 'green' nuance in the wine, with a level of 7 ng/L IBMP not perceived as 'green', but just causing a slight loss of fruitiness. As the panellists in this experiment were all very experienced, having worked on smoke taint and other aroma projects on a regular basis, it was felt that the lower levels were justified.

Table 2. Samples prepared for five compounds at peri- and sub-detection threshold

Sample	Code	Description	Spike (µg/ L)
DSW_1	Control 1	Dearomatised Shiraz wine	0
DSW_2	Control 2	Dearomatised Shiraz wine	0
Gu_DT	1A(ODT)	Factor 1 (guaiacol)	23
Gu_BeDT	1B below ODT	Factor 1 (guaiacol)	15
*o*Cr_DT	2A(ODT)	Factor 2 (*o*-cresol)	62
*o*Cr_BeDT	2B below ODT	Factor 2 (*o*-cresol)	40
4-EP_DT	3A(ODT)	Factor 3 (4-EP)	605
4-EP_BeDT	3B below ODT	Factor 3 (4-EP)	400
IBMP_DT	4A (75% of ODT)	Factor 4 IBMP	10 ng/L
IBMP_BeDT	4B (~50% ODT)	Factor 4 IBMP below ODT	7 ng/L

2.2.2 Preparation of 'binary'/ combination samples

This study focused on the separate sensory effects of the three volatile phenols and IBMP in binary mixtures, and therefore wine samples were spiked with only two of each of the four compounds, following a D-optimal fractional statistical design. This design, generated by "R" (R Development Core Team, Austria) software which minimizes variability and takes into account qualitative and quantitative factors. This allows a smaller sample set, and avoid the necessity of carrying out a full factorial analysis, which is time consuming, expensive and risky when dealing with descriptive analysis and sensory panels.

Table 1. 'Binary' samples spiked at threshold (A) and subthreshold (B) levels (µg/L unless otherwise specified)

sample	Compound 1	level	spike	Compound 2	level	spike
1	Guaiacol	15	B	*o*-cresol	62	A
2	Guaiacol	23	A	*o*-cresol	40	B
3	Guaiacol	15	B	4-EP	605	A

4	4-EP	605	A	*o*-cresol	40	B
5	Guaiacol	23	A	4-EP	400	B
6	Guaiacol	15	B	4-EP	400	B
7	4-EP	400	B	*o*-cresol	62	A
8	4-EP	400	B	*o*-cresol	40	B
9	Guaiacol	23	A	IBMP	7 ng/L	B
10	Guaiacol	15	B	IBMP	7 ng/L	B
11	*o*-cresol	62	A	IBMP	7 ng/L	B
12	IBMP	7ng/L	B	*o*-cresol	40	B
13	4-EP	400	B	IBMP	7ng/L	B

2.3 Panel selection

The panel consisted of 11 judges, all non-smoking females between the ages of 24 and 60. Judges had previous experience in the use of descriptive analysis, and experience in smoke taint evaluation in wine. Most of the panellists also took part in a triangle test to test sensitivity and confirm odour detection thresholds for these compounds (McKay *et al.*, 2018) and therefore already had some familiarity with the compounds under investigation.

2.4 Sensory training

Training consisted of three sessions. During the first session, the judges received all the samples, as well as a range of reference standards adapted from Noble *et al.*, (1987). The purpose of this session was to familiarise the judges with the samples and descriptors used during this part of the study. The compounds were profiled using descriptive analysis (DA) according to the general descriptive method (Lawless & Heymann, 2010). During the subsequent training sessions, 30 descriptors were generated for the spiked and control wines using descriptive analysis. These descriptors were ‘berries’ and ‘sweet-associated/vanilla’, ‘jammy’, ‘floral/violets’, ‘prunes’, ‘plums’ ‘burnt sugar’, ‘tobacco’, ‘*rooibos* tea’, ‘liquorice’, ’green pepper’, ‘herbaceous’, ‘earthy/ dusty/ potato skin’, ‘leather’, ‘barnyard’, ‘smoky’, ‘ashtray’, ‘pencil shavings’ ‘medicinal/Elastoplast™’, ‘tar’, ‘Doom’ (insect spray), ‘soy sauce’, ‘balsamic’, ‘alcohol’ ‘black pepper’, ‘mouldy/ musty’, ‘cooked vegetables’ and 'acetone'. These descriptors were used for testing the 'single' spiked compounds, but were further rationalised during subsequent panel sessions for the 'binaries' as the panel found the list far too extensive under testing conditions.

Attributes used in the final binary testing conditions were reduced during consensus training to the following15 descriptors: 'mouldy/musty', 'plums', 'soy sauce', 'medicinal/Elastoplast™', 'herbaceous/green', 'floral/violets', 'tobacco', 'pencil shavings', 'earthy/dusty/ potato skin', 'sweet-associated', 'leather/ barnyard', 'berries', 'prunes/raisins', 'tar/BR', 'chemical/plastic'.

2.5 Sensory testing

Individual (single) compounds (guaiacol, *o*-cresol, 4-EP, IBMP) were tested at at sub- and peri-threshold concentrations in the partially de-aromatised base wine in a sensory laboratory equipped with individual booths with standard artificial daylight lighting and temperature control at 20 ± 1°C. Wines were coded with unique three digit codes presented to judges in black ISO 3591:1977 standard glasses and covered with plastic lids. The order of samples was 'counterbalanced' across individuals, by changing the presentation order, as recommended by Lawless & Heymann (2010) and presented monadically according to William Latin Square design. All glasses were prepared one hour before serving to allow for temperature and headspace equilibration. Testing for 'singles' (individual compounds) was conducted over three sessions (triplicates of different combinations), each session consisting of wine spiked with four different compounds at two concentrations, and two controls. Judges were asked to evaluate the twelve samples orthonasally (i.e., by sniffing), and not to taste, as the study was concerned only with odour thresholds and aroma effects, and not with palate effects. Communication was not allowed between the judges for the duration of the test. Different compounds were not given in a specific order but randomised across all compound and all levels. To minimise tiredness of the sense organs, the set of twelve wines was divided in sub-sets of 4 samples and judges were asked to rest for fifteen minutes between each sub-sets.

Testing for 'binary' samples (compounds in combination) followed the partial factorial design outlined in Section 2.2 (Table 3). Each test was conducted following the same rules as outlined above for singles compounds. Judges were therefore presented with thirteen samples spiked following the design which encompassed sub- and per-threshold concentrations.

2.6 Data analysis

Sensory data generated by Descriptive Analysis was examined by analysis of variance (ANOVA) to determine which descriptors have significantly different intensities between products, and to account for judge effects. The one-way and two-way ANOVAs were performed using Statistica, Version 12 (StatSoft, Tulsa, USA) and the means were separated using Stats-Fisher's LSD test (different letters denoting significant differences at $p \leq 0.05$). Least Squares Means (LSM) diagrams for attributes and compounds were generated from the two-way ANOVAS. Principal Component Analysis (PCA) biplots were compiled from datasets for individual compound samples, and binary samples using PanelCheck to help explain variance (Lawless & Heymann, 2010). All quoted uncertainty is the standard deviation of three replicates of one treatment.

3. Results and discussion

As there is limited information in the literature on the perception of these compounds in various matrices, the effects of the individual compounds on aroma in the chosen matrix needed to be established by the panel before perceptions of binary mixtures could be characterised.

3.1 Attributes in red wine spiked with single compounds

3.1.1 General observations

The descriptors in the samples spiked with individual compounds included 'plums', 'pencil shavings', 'smoky', 'soy sauce', 'black pepper', 'mouldy/ musty' and 'cooked vegetables'. The PCA biplot of the sensory results of the individual compounds spiked at their ODTs and subthreshold levels in dearomatised Shiraz wine is shown in Figure 1.

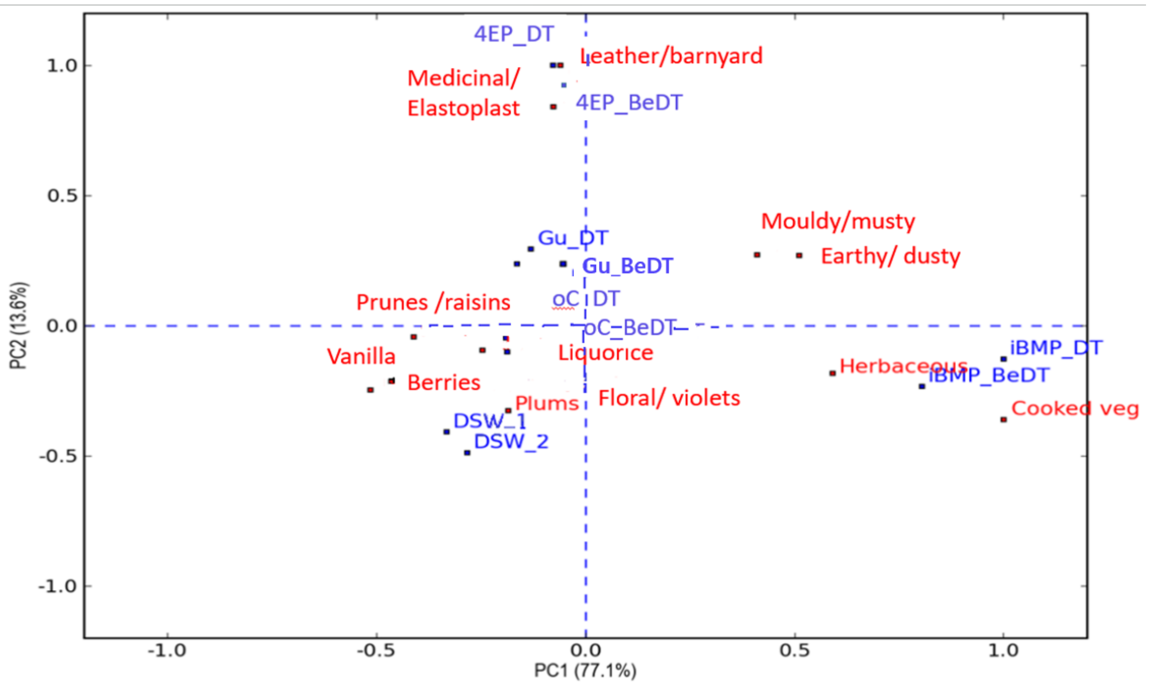


Figure 1: PCA biplot of the sensory results of attributes associated with individual compounds (guaiacol (Gu), o-cresol (oCr), 4-EP, IBMP) in dearomatised Shiraz Wine (DSW) at peri (DT) - and subthreshold (BeDT) levels.

Attributes associated with the five sample sets spiked with individual compounds at sub-and prei-threshold levels separate into three major groupings. Over 90% of the variance in the data is explained by the first two principal components, with separation along PC1 (77%) contrasts samples rated relatively highly in fruity/sweet aromas with those rated highly in green-related attributes, located on the right of the plot. The controls (DSW_1 and DSW_2) are strongly associated with 'berries', 'vanilla', 'floral/violet' and 'plum' attributes. Samples containing *o*-cresol are most closely associated with the descriptors 'prunes/raisins', 'alcohol' and 'liquorice', as is the sample containing guaiacol at sub-threshold levels. The samples containing 4-EP are associated with 'leather/barnyard' and 'medicinal/Elastoplast™' attributes. There is some separation (13.6%) along PC2 between 'leather/barnyard/medicinal' attributes and the 'sweet/fruity' cluster in the center of the PCA. Guaiacol and *o*-cresol do not seem to have as much effect, which contradicts what has been found previously in the literature (Parker *et al.*, 2012). Both 4-EP and IBMP at 65% of their accepted literature values caused negative olfactory effects that were similar to their full ODT values, with a small separation towards the control samples for their lower values. This was unexpected, as in the benchtop screening, the lower levels did not have a marked effect. It may be the case that with training and exposure, the panel became sensitised to the lower levels of the compounds, and were able to detect small differences. This would be in accordance with the theory of learned odour-object encoding put forward by various authors (Wilson, 2005; Wilson & Stevenson, 2010; Wilson & Sullivan, 2011).

3.1.2 Individual compound effects on sweet and fruity attributes

As the impact of various off flavours on the perception of the fruity character of wine has been described previously, the effect of the four compounds on the fruity/sweet character was investigated. Guaiacol is known to impart a smoky, burnt character and exhibits the lowest aroma detection threshold of the VPs (Boidron et al., 1988). It is generally the most abundant of these compounds detected in smoke-affected wines (Kennison et al., 2007; Kennison, 2013), and is considered to be an important indicator for smoke-related taints. In the dearomatised red wine matrix in this study, guaiacol showed a tendency to increase the perception of 'berries' aroma even at subthreshold level (Figure 2i), but did not have any significant effect on the 'smoky' attribute, which is contrary to what has been found by previous workers (Atanasova, et al., 2005). The presence of o- cresol at sub- and peri-threshold concentrations increased the perception of the 'sweet-associated' attribute (Figure ii) (p=0.02). 4-EP had an effect across a range of fruity attributes, significantly increasing perception of positive attributes 'berries', 'sweet-associated' and 'prunes/raisins' (Figure 2 iii) (p=0.06), which was unexpected. IBMP at peri- and subthreshold levels had the effect of significantly decreasing all the following attributes in the dearomatised red wine compared to clean controls: 'berries' (p=0.001),' floral/violets'(p=0.005) (Figure 2iv), 'prunes/raisins' (p=0.004), 'sweet-associated' (p=0.001), 'tobacco' (p=0.01). This is in accordance with the literature (Allen *et al.*, 1996) which have shown IBMP to have a marked effect on fruity notes in wine.

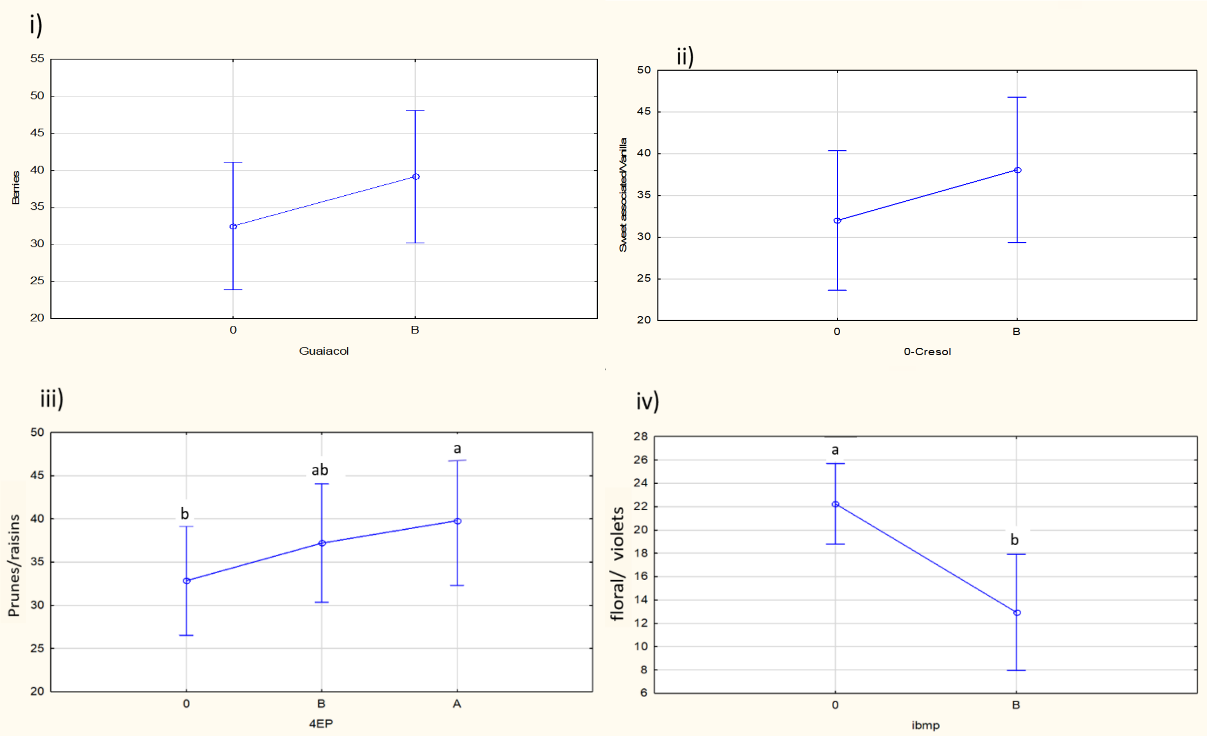


Figure 2. LSM Type III decomposition diagrams of effect on selected sweet and fruity attributes by compounds (0= control wine; B= below or subthreshold, A= at or peri-threshold). Vertical bars denote 0.95 confidence intervals. i) guaiacol (p=0.04); ii) o-cresol (p=0.02); iii) 4-EP (p=0.06); iv) IBMP (p=0.005).

3.1.3 Individual compound effects on other attributes

'*Herbaceous/green attributes'*: In the PCA (Figure 1) IBMP can be seen to be strongly associated with 'herbaceous' and 'cooked vegetable' (both generally acknowledged as 'green') attributes, as would be expected from previous studies (Allen *et al.*, 1996). There was no contribution by other compounds to increasing this descriptor (Figure 3), and there was no difference in perception if IBMP was peri-or subthreshold. The attribute 'cooked vegetables' was perceived in 31 % of the 'singles' samples, and was also strongly correlated to IBMP present at and below detection ($p<0.001$), as was 'herbaceous/green' ($p=0.001$). This attribute was perceived to decrease significantly ($p=0.04$) in the presence of guaiacol at sub- and peri- threshold levels (Figure 3i). The 'herbaceousness' attribute was also decreased by the presence of *o*-cresol (Figure 3 ii) ($p=0.04$). *o*-Cresol has been shown by Parker *et al.*, (2013) to add to 'burnt', 'smoky', 'medicinal' and 'tar-like' attributes, but its role in reduction of green attributes has not been previously shown.

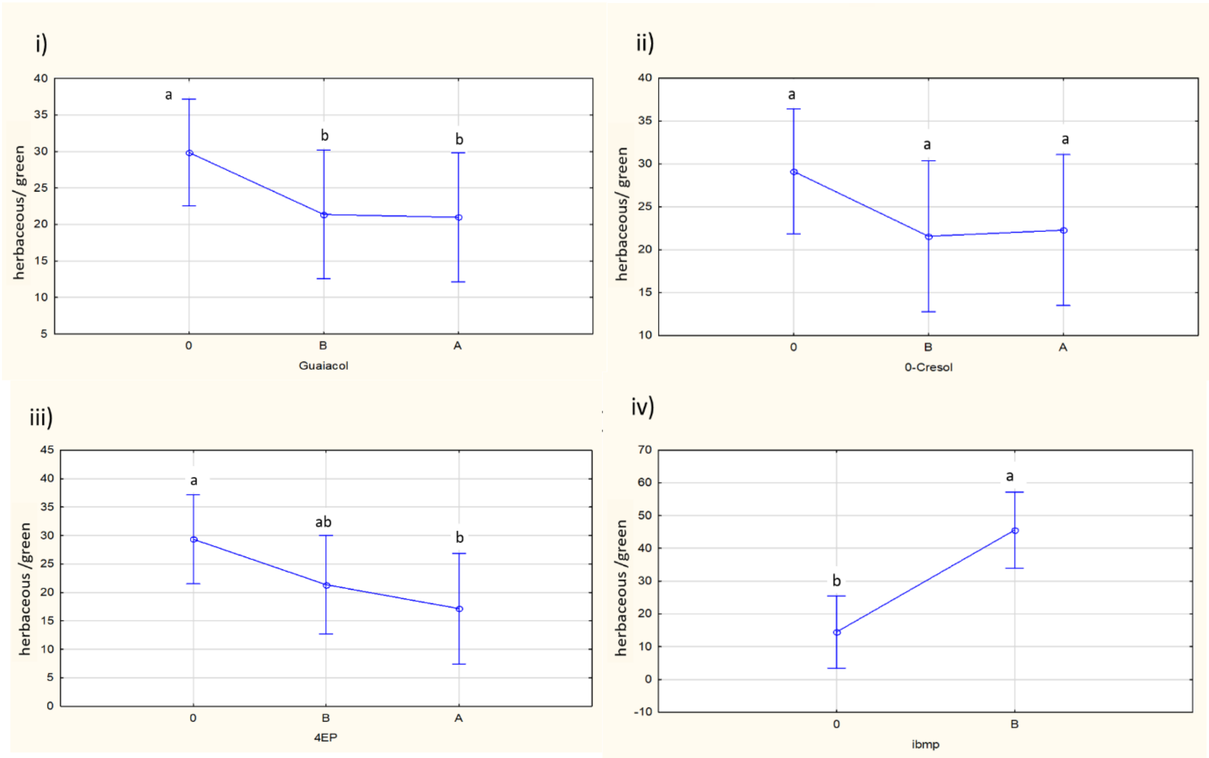


Figure 3. LSM Type III decomposition diagrams for effect on the ‘herbaceous/green’ attribute by compounds (0= control wine; B= below or subthreshold, A= at or peri-threshold). Vertical bars denote 0.95 confidence intervals i) guaiacol (p=0.09); ii) o-cresol (p=0.04); iii) 4-EP (p=0.06); iv) IBMP (p=0.001).

‘Earthy/dusty/potato skin’ attributes (Figure 4): The perception of ‘earthy/dusty’ attribute was significantly decreased by the presence of guaiacol at DT (p=0.04) (Figure 2ii). The ‘earthy/dusty’, ‘savoury’, ‘leather/barnyard’ attributes were decreased by the presence of *o*-cresol (Figure 3 ii-iv). This result was unexpected, as *o*-cresol has been shown by Parker *et al.*, (2013) to add to ‘burnt’, ‘smoky’, ‘medicinal’ and ‘tar-like’ attributes.

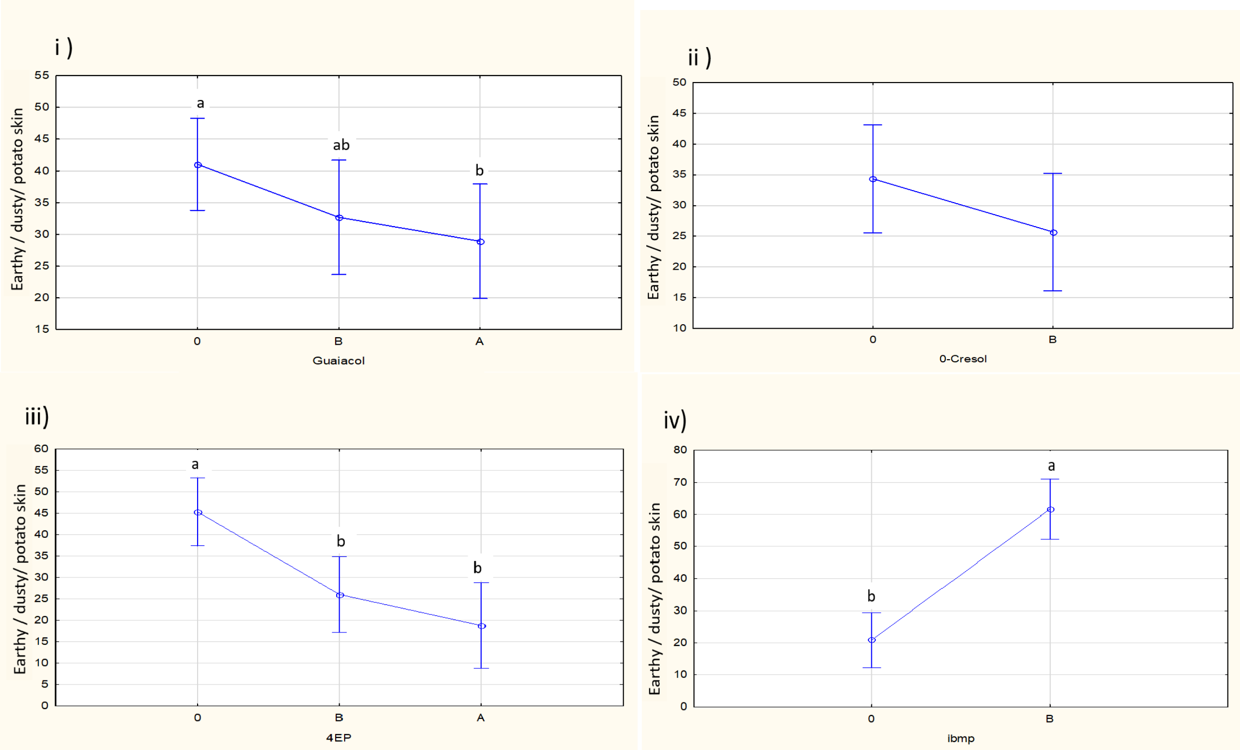


Figure 4. LSM Type III decomposition diagrams for the effect of compounds on the 'earthy/ dusty/ potato skin' attributes (0= control wine; B= below or subthreshold, A= at or peri-threshold). Vertical bars denote 0.95 confidence intervals. i) Guaiacol (p=0.02); ii) o-cresol (p=0.09); iii) '4-EP (p<0.001); iv) IBMP (p<0.001).

'Medicinal /Elastoplast™' and 'leather/barnyard' attributes: In the PCA (Figure 1) 'leather/barnyard' and 'medicinal/Elastoplast™' attributes are associated strongly with 4-EP, separating from the rest of the attributes, as would be expected from previous descriptions of the odour qualities imparted to wine by this compound (Petrozziello *et al*., 2014). These attributes are generally perceived as negative to the quality of wine. The perception of the 'medicinal/Elastoplast™' attribute was slightly increased by the presence of guaiacol (p=0.04) (Figure 5i) and 4-EP (Figure 5iii) at sub- and peri-threshold levels. Perception of 'medicinal/ Elastoplast' (p=0.08) and 'leather/barnyard' (p=0.005) attributes is shown in Figures 5 ii) and iv) were significantly increased by subthreshold and peri-threshold concentrations of 4-EP. Surprisingly, 'herbaceous/green pepper', 'earthy/ dusty' and 'ashtray' were significantly decreased by 4-EP at both concentrations.

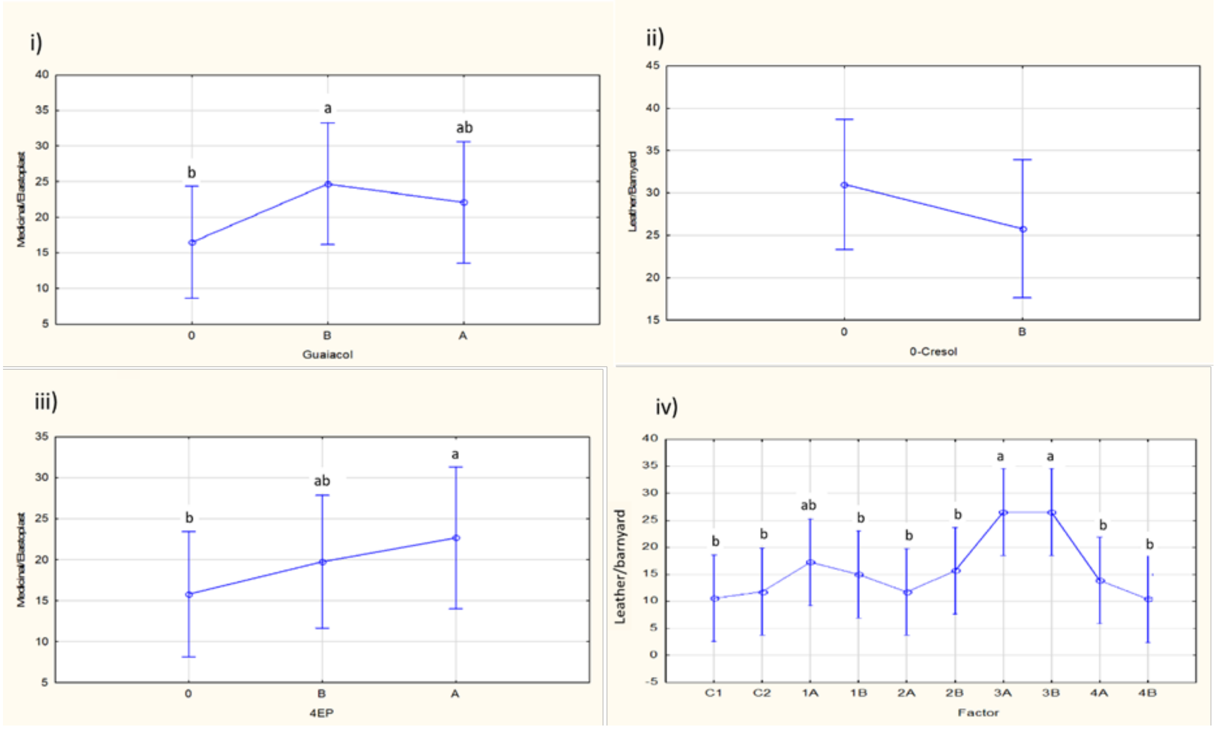


Figure 5. LSM Type III decomposition diagrams for 4-EP effect on ‘medicinal/Elastoplast’ and ‘leather/barnyard’ (0= control wine; B= below or subthreshold, A= at or peri-threshold). Vertical bars denote 0.95 confidence intervals. i) guaiacol ($p=0.04$); ii) o-cresol ($p=0.09$); iii) 4-EP ($p<0.09$); iv) All compounds on ‘leather/barnyard’ ($p=0.005$) showing controls (C1/2) and spiked compounds guaiacol (1A/B), o-cresol (2A/B), 4-EP (3A/B) and IBMP (4A/B).

IBMP at peri- and subthreshold levels had the largest effect of all of the four compounds in the study, significantly decreasing all the following attributes: medicinal/Elastoplast™’ ($p=0.005$), ‘plastic/chemical’ ($p=0.06$) and ‘savoury’ ($p=0.04$) and ‘tobacco’ ($p=0.01$) in dearomatised red wine compared to unspiked controls. It also significantly increased the perception of ‘mouldy/musty’ ($p=0.04$) at both peri- and subthreshold levels compared to other compounds.

3.2 Attributes and interactions perceived in binary mixtures

It was shown that individual compounds in the de-aromatised red wine matrix had some interesting effects, and this trend carried through to the perceptions of interactions in binary mixtures.

The PCA biplot in Figure 6 shows overall trends for triplicates of spiked compounds. Each triplicate of wines has an associated code, with the first position occupied by guaiacol at either 0 (not present), B (below detection), or A (at threshold) levels. The second position is *o*-cresol, the third is 4-EP and the fourth is IBMP. Thus 0B0A contains no guaiacol, below detection levels of *o*-cresol, no 4-EP and above detection levels of IBMP. The PCA shows that 89% of the variation within the dataset is explained by PC1, the separation of wines containing binary spikes of volatile phenols combined with low levels of IBMP leading to attributes ‘earthy/dusty’, ‘ashtray’ and ‘herbaceous/green’ attributes from control wines, and those containing binary combinations of volatile phenols only. This is an interesting result, given the anecdotal evidence that South African wines (particularly the cultivars Merlot Noir and Cabernet Sauvignon) may manifest a green off-flavour despite containing very low levels of IBMP or the other pyrazines.

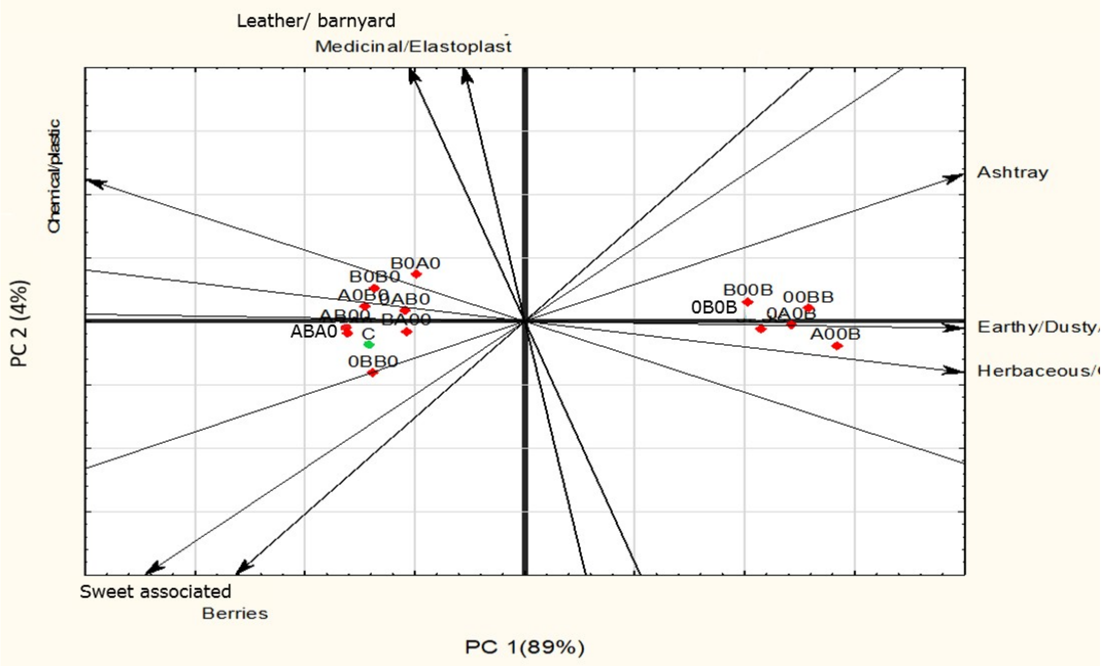


Figure 6. PCA biplot of the sensory results of attributes of control wine (C in green), and samples spiked with binary combinations of the three VPs: guaiacol (position 1), o-cresol (position 2), 4-EP (position 3), and IBMP (position 4) in dearomatised Shiraz wine at 0 peri (A) and subthreshold (B) levels.

When guaiacol and *o*-cresol are present together in solution, the perception of 'floral/violets' and 'sweet- associated' are increased significantly (Figure 7:i and ii), which is interesting given that the descriptors for these two phenols include 'burnt', 'smoky', 'phenolic' and 'medicinal' (Chatonnet, *et al*., 1992; Parker, *et al.,* 2013, De Vries, *et al*., 2016). The 'berries' attribute (detected in 49% of samples) was increased by guaiacol and *o*-cresol at both detection levels, but decreased by IBMP (p<0.001). This finding is in agreement with Hein *et al.*, (2009) who observed a decrease in fruity aroma perception of wine, if green (bell) pepper aroma was present. Guaiacol and *o*-cresol also interacted to increase the perception of the 'floral/violets' attribute significantly (p=0.02) at peri-threshold levels. Perceptions of 'earth/dusty', and 'herbaceous/ green' attributes in the wine are decreased by these two volatile phenols in combination (Figure 7 iii and iv).

IBMP again has the largest effect on perceived attributes in combination with other compounds. It significantly decreases 'floral/violets', both at peri- and subthreshold levels (p=0.005). Guaiacol and *o*-cresol interact at peri- and subthreshold levels to decrease the 'herbaceous/ green' attributes, but if 4-EP and IBMP are present together at DT levels, there is a significant increase in 'herbaceous/green' attribute, with 4-EP seeming to magnify the effect of IBMP. The 'earthy/ dusty/ potato skin' attribute was perceived in both singles and binaries. IBMP had the most significant effect on this attribute, increasing this descriptor significantly (p<0.001), even in combination with 4-EP. 4-EP significantly decreased the perception of 'earthy/dusty' at threshold levels unless in combination with IBMP. The descriptor was also significantly decreased by guaiacol and *o*-cresol in combination, at detection threshold levels. This effect only happened when both VPs were present. The 'leather/barnyard' attribute was influenced by the presence of 4-EP, but there did not seem to be a difference between peri- or subthreshold levels on the perception. It was not affected by other compounds. The 'medicinal/ Elastoplast™' attribute was shown to be perceived more strongly in mixtures containing guaiacol and *o*-cresol. There is a significant increase at peri-threshold levels for 4-EP on this attribute, and IBMP causes decreases in its perception, but this is not significant.

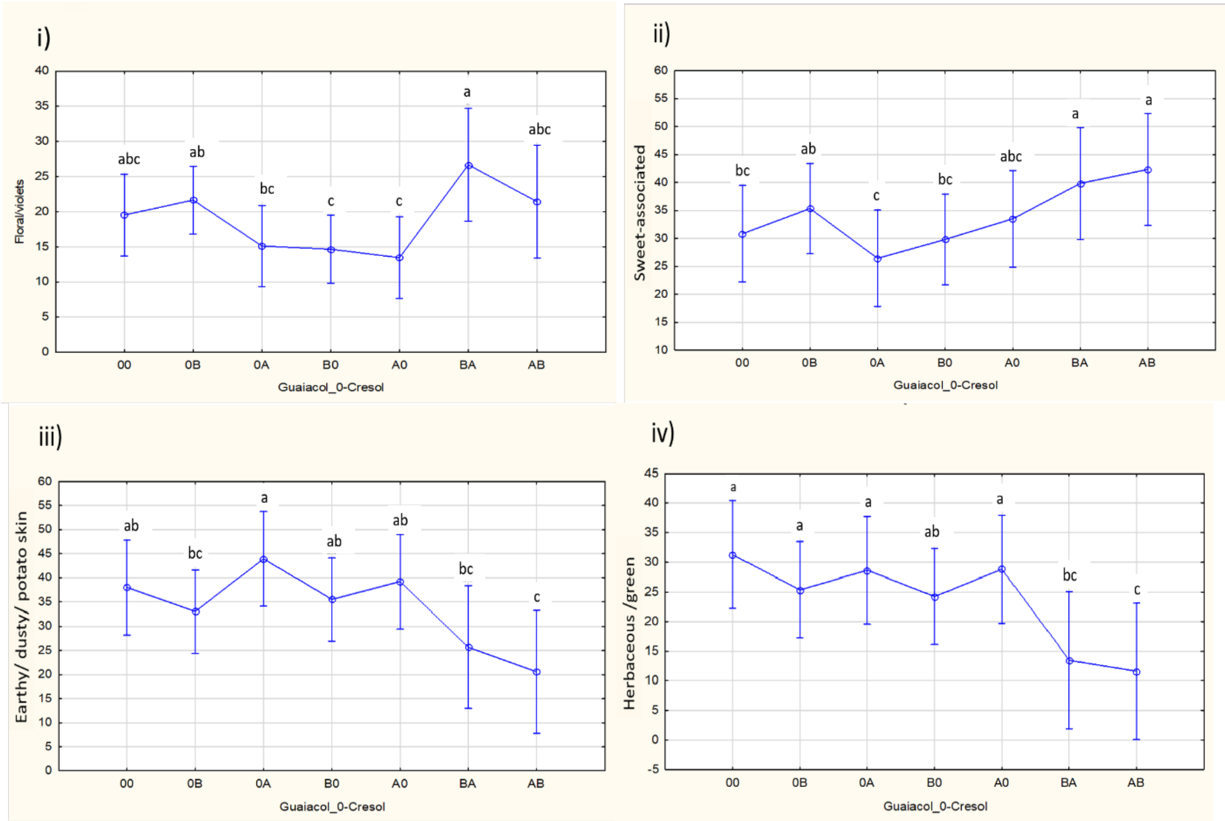


Figure 7. Treatment LS Means Type III decomposition diagrams for binary solutions of guaiacol and o-cresol showing effects on perception of selected attributes i) 'floral/violets' attribute (p= 0.04); ii) 'sweet-associated' ((p=0.004); iii) 'Earthy/dusty (p=0.02) and iv) 'herbaceous/green (p=0.01). 'A' denotes peri-threshold level spike, 'B' denotes subthreshold spike.Vertical bars denote 0.95 confidence intervals.

The attributes 'savoury', 'tar/BR', 'ashtray' and 'plastic/chemical' were only perceived in the samples with binary spikes. These descriptors were not used in the description of samples with only individual compounds. When guaiacol and 4-EP (Figure 8 i) were combined in solution, the attribute 'tar/ burnt rubber (BR)' was used, but this attribute was not used to describe perceptions of individual compounds in solution, or to describe the base wine. 4-EP at peri-detection threshold level, combined with guaiacol at subthreshold level will increase the intensity of perception of this attribute significantly. This is in accordance with findings by Panzeri (2013), who demonstrated that the attribute 'burnt rubber' only appeared in spike solutions of unwooded Pinotage where 4-EP was present in combination with phenol and *o*-cresol, and *o*-cresol was responsible for 'smoky- ashy' attributes when in combination with 3,4-dimethylphenol and phenol.

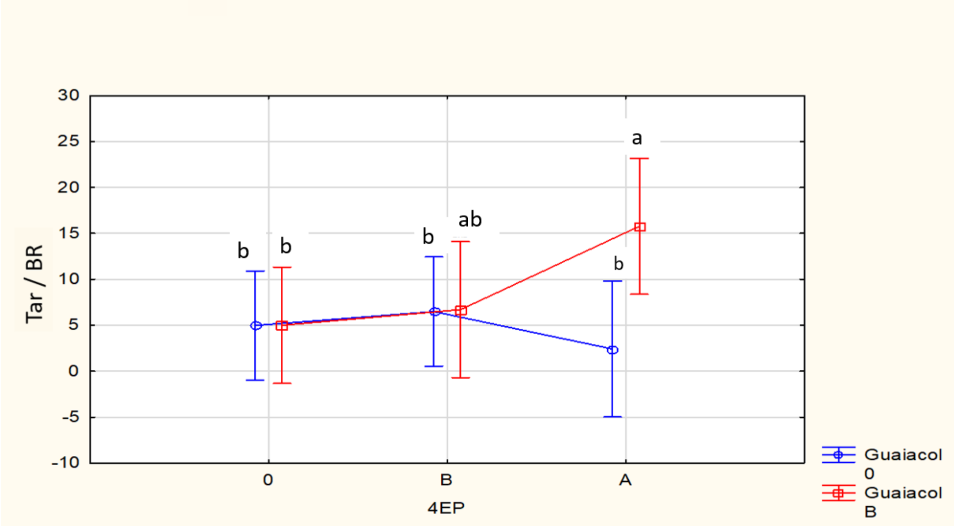


Figure 8. LS Means Type III decomposition diagrams for a binary solution of volatile phenols 4-EP and guaiacol showing the 'tar/ BR' attribute (p=0.06). 'A' denotes peri-threshold level spike, 'B' denotes subthreshold spike, and 0 indicates control/ no added compound. Vertical bars denote 0.95 confidence intervals.

Without the effect of 4-EP, the 'tar/BR' attribute decreases, so the observation by Panzeri (2013) that 4-EP is a major contributor to 'burnt rubber' if other phenols are present seems substantiated. The presence of low levels of IBMP has a marked effect on binary combinations (Figure 9 i-iv). When volatile phenols are in combination with IBMP, there is a significant variation in attributes compared to the control, and when VPs are in combination together. As can be seen in Figure 9, combinations of IBMP with guaiacol, 4-EP, and *o-* cresol led to significant increases (p<0.001) in 'earthy/dusty/potato skin', 'herbaceous/green' and 'ashtray' attributes. It significantly decreased (p<0.001) the 'berries' attribute.

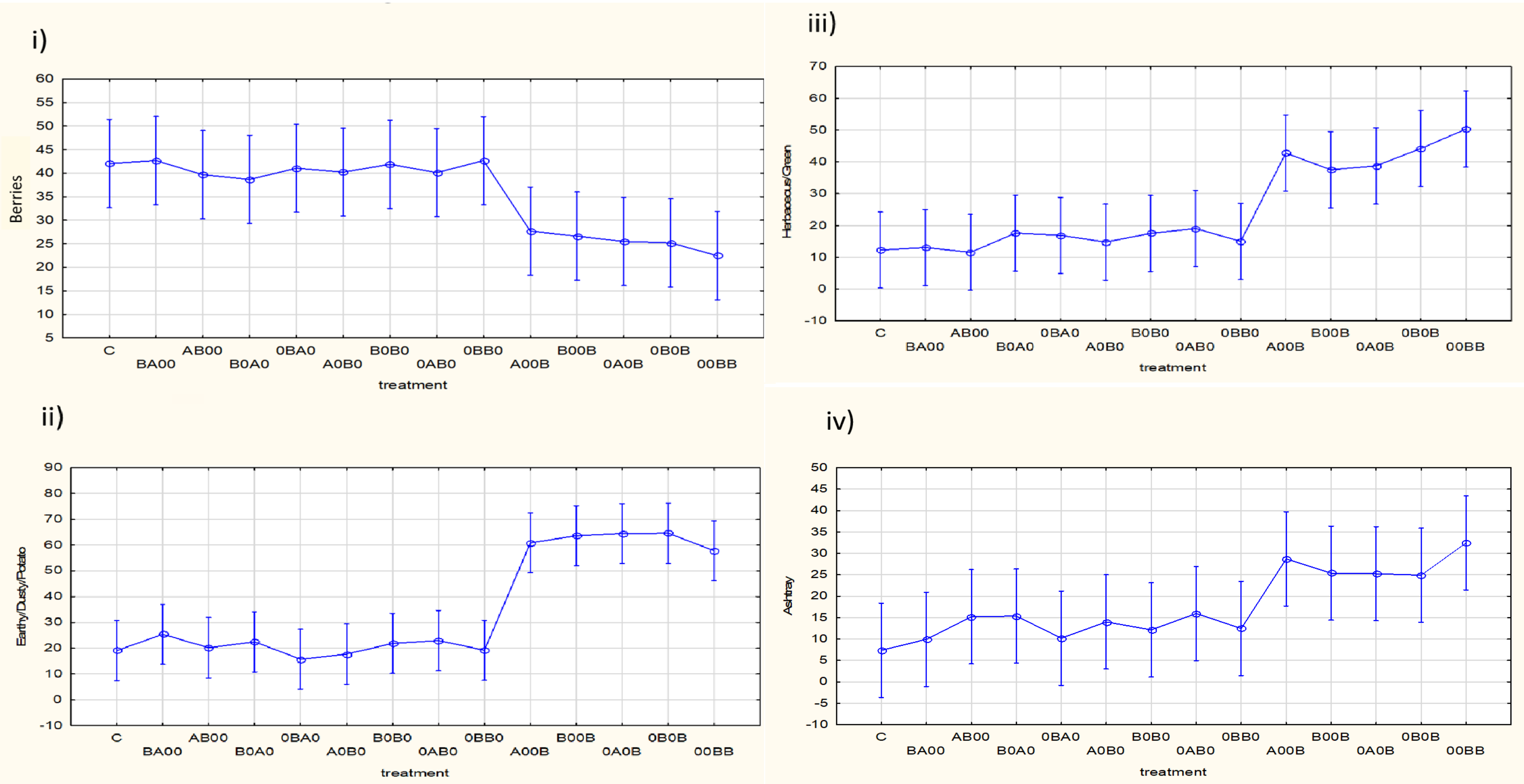


Figure 1. Treatment LS Means Type III decomposition diagrams for binary solutions of volatile phenols with IBMP showing effects on perception of selected attributes i) 'berries' attribute (p<0.001); ii) 'Earthy/dusty (p<0.001); iii) 'herbaceous/green (p<0.001) iv) 'Ashtray' (p<0.001). A/B denote peri-or subthreshold level for each compound: (guaiacol, o-cresol, 4-EP and IBMP in that order). C denotes control samples. Vertical bars denote 0.95 confidence intervals.

The perception of ‘berries’ (Figure 9i) (p=0.001), as well as other positive descriptors ‘prunes/ raisins’, ‘sweet- associated’, ‘tobacco’, and ‘floral/violets’ decreased when volatile phenols and IBMP were present together, with 4-EP having marginally more effect than the other phenols. The ‘plastic/chemical’ and ‘medicinal/Elastoplast™’ attributes also decreased, which is interesting given that the latter attribute increases significantly when 4-EP is present on its own in solution. The attributes ‘herbaceous/green’ (p<0.001) (Figure 9 iii), ‘plastic/chemical’ (p=0.03), ‘earthy/ dusty/potato skin’) (p<0.001) (Figure 9 ii and ‘ashtray’ (p<0.001) (Figure 9 iv) were all perceived to intensify the effect that IBMP had when present on its own, although this is not significantly different from the perception of IBMP as a single compound in a red wine matrix.

4. Conclusions

In order to elucidate olfactory perceptual interactions between four compounds associated with complex effects, and some off-flavours (guaiacol, *o*-cresol, 4-EP, and IBMP) in wine, a partially de-aromatised red base wine was first spiked with individual compounds, at two different levels (peri-threshold and subthreshold) for each compound, and evaluated by a trained panel using DA. The panel was subsequently presented with binary mixtures of compounds at subthreshold and peri-threshold levels in a partial factorial statistical design, and asked to evaluate the aroma of these mixtures. Results indicated that certain attributes were perceived by panellists only in the samples with individual compounds. These included ‘cooked vegetables’, ‘black pepper’, ‘mouldy/musty’, ‘pencil shavings’, ‘smoky’, ‘soy sauce’ and ‘plums’. PCAs of the DA results indicated that in this matrix both 4-EP and IBMP at 65% of their accepted literature values caused negative olfactory effects that were similar to their full ODT values, with slight separation towards the control samples for their lower values. This was unexpected, as in the benchtop screening, the lower levels did not have a marked effect compared to the base wine.

Binary systems generated different descriptors from single compound systems. The aroma attributes ‘ashtray’, ‘plastic/chemical’, ‘Tar/BR’ and ‘savoury’ were perceived only in binaries, and were not necessarily associated only with detection threshold levels. In some cases, the subthreshold level of a compound was sufficient to induce significant interactions and effects. The attribute ‘ashtray’, known to be associated with guaiacol and smoke taint, was in this case affected significantly by IBMP at peri-threshold levels. The 4-EP-IBMP combination seemed to enhance the effect of IBMP. Although the perception of the ‘savoury’ attribute did not seem to be affected by any specific compound or combination, this attribute was only detected in binary combinations. Perception of the ‘plastic/ chemical’ attribute also did not show strong associations with particular compounds, with only 4-EP and IBMP together appearing to reduce this attribute.

The ‘tar / BR’ descriptor was also only used to describe binary samples. Guaiacol and 4-EP in combination in the red wine matrix at even subthreshold levels were shown to produce this attribute.

4-EP gives this tarry attribute at DT levels in combination with guaiacol, but does not seem affected by the presence of *o*-cresol which was unexpected given that guaiacol and *o*-cresol have similar descriptors.

Results indicated that some binary mixtures in red wine do not give olfactory results that can be predicted from the attributes of the same single compounds in the matrix. It also seemed to be the case, as theorised by previous workers, that exposure, experience and learning led the panellists in this study to become sensitised to the lower levels of the volatile phenols, and they were able to detect small differences in systems containing these compounds, as would be predicted by 'odour-object' encoding theory and peripheral level interactions. This work thus adds to the understanding of the perception of olfactory qualities of binary mixtures, and sheds light on the behaviour and impact of low levels of volatile phenols and IBMP in wine, and helps to inform the training of panellists and judges in wine evaluation.

Acknowledgments: Winetech and THRIP (NRF) acknowledged funding associated with volatile phenol projects. Martin Kidd designed and carried out the statistical analysis.

Author Contributions: Marianne McKay, Astrid Buica and Florian Bauer conceived and designed this study; Valeria Panzeri led the sensory panel, assisted where appropriate by Marianne McKay; Martin Kidd conceived the statistical design. Marianne McKay carried out all the practical work for the project and wrote the paper, which was revised and edited by Astrid Buica, Florian Bauer, and Valeria Panzeri.

REFERENCES

Allen, M.S., Lacey, M.J., *et al*., 1996. Existence of different origins for Methoxypyrazines of grapes and wines In: G.R. Takeoka, R. Teranishi, P.J. Williams, & A. Kobayashi (eds). Biotechnol. Improv. Foods Flavors. American Chemical Society, Washington DC 220–227.

Atanasova, B., Thomas-Danguin, T., *et al*., 2005. Perception of wine fruity and woody notes: influence of peri-threshold odorants. Food Qual. Prefer. 16, 6, 504–510.

Atanasova, B., Thomas-Danguin, T., *et al*., 2005. Perceptual Interactions in Odour Mixtures: Odour Quality in Binary Mixtures of Woody and Fruity Wine Odorants. Chem. Senses 30, 3, 209–217.

Bearak, B., 2009. A Whiff of Controversy and South African Wines New York Times (New Yrok) 28 June. Burdach, K.J., Köster, E.P., *et al*., 1985. Interindividual differences in acuity for odor and aroma. Percept. Mot. Skills 60, 3, 723–730.

Chaput, M.A., El Mountassir, F., *et al*., 2012. Interactions of odorants with olfactory receptors and receptor neurons match the perceptual dynamics observed for woody and fruity odorant mixtures. Eur. J. Neurosci. 35, 4, 584–597.

Chatonnet, P., Dubourdie, D., *et al*., 1992. The origin of Ethylphenols in wines. J. Sci. Food Agric. 60, 2, 165–178.

Czerny, M., Brueckner, R., *et al*., 2011. The influence of molecular structure on odor qualities and odor detection thresholds of volatile alkylated phenols. Chem. Senses 36, 6, 539–553.

Hein, K., Ebeler, S., *et al*., 2009. Perception of fruity and vegetative aromas in red wine. J. Sens. Stud. 24, 3, 441–455.

Heyns, E., 2014. The Green South African palate - When does mint become eucalyptus or even downright weedy? - Wineland Magazine Available at http://www.wineland.co.za/the-green-south-african-palate-when- does-mint-become-eucalyptus-or-even-downright-weedy/.

Kennison, K., 2013. Effect of smoke in grape and wine production. Gov. West. Aust. Dep. Agric. Food Bull. Bulletin 4.

Lawless, H.T. & Heymann, H., 2010. Sensory Evaluation of Foods: Principles & Practices. (2nd ed.). Springer Science Business Media LLC, New York.

Lawless, H. & Heymann, H., 2010. Measurement of Sensory Thresholds. (2nd Ed.). Springer Science & Business Media (Food Science Text Series), New York.

McKay, M., Bauer, F., *et al*., 2018. Testing the Sensitivity of Potential Panelists for Wine Taint Compounds Using a Simplified Sensory Strategy. Foods 7, 11, 176.

Munch, D., Schmeichel, B., *et al*., 2013. Weaker Ligands Can Dominate an Odor Blend due to Syntopic Interactions. Chem. Senses 38, 4, 293–304.

Noble, A.C., Arnold, R.A., *et al*., 1987. Modification of a Standardized System of Wine Aroma Terminology. Am. J. Enol. Vitic 38, 2.

Parker, B.M., Baldock, G., *et al*., 2013. Seeing through smoke. Wine Vitic. J. 42–46.

Parker, M., Osidacz, P., *et al*., 2012. Contribution of several volatile phenols and their glycoconjugates to smoke-related sensory properties of red wine. J. Agric. Food Chem. 60, 10, 2629–2637.

Perry, D.M. & Hayes, J.E., 2016. Effects of matrix composition on detection threshold estimates for Methyl Anthranilate and 2-Aminoacetophenone. Foods 5, 2, 35–45.

Romano, A., Perello, M.C., *et al*., 2009. Sensory and analytical re-evaluation of “Brett character” Food Chem. 114, 1, 15–19.

Rospars, J.-P., Lansky, P., *et al*., 2008. Competitive and Noncompetitive Odorant Interactions in the Early Neural Coding of Odorant Mixtures. J. Neurosci. 28, 10, 2659–2666.

Roujou de Boubée, D., Van Leeuwen, C., *et al*., 2000. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red bordeaux and loire wines. Effect of environmental conditions on concentrations in grapes during ripening. J. Agric. Food Chem. 48, 10, 4830–4.

Sell, C.S., 2006. On the Unpredictability of Odor. *Angew. Chemie* Int. Ed. 45, 38, 6254–6261.

Tempere, S., Schaaper, M.H., *et al*., 2016. The olfactory masking effect of ethylphenols: Characterization and elucidation of its origin. Food Qual. Prefer. 50, 135–144.

Thomas-Danguin, T., Sinding, C., *et al*., 2014. The perception of odor objects in everyday life: a review on the processing of odor mixtures. Front. Psychol. 5, June, 1–18.

de Vries, C.J., Mokwena, L.M., *et al*., 2016. Determination of Volatile Phenol in Cabernet Sauvignon Wines, Made from Smoke-affected Grapes, by using HS-SPME GC-MS. South African J. Enol. Vitic. 37, 1, 15–21.

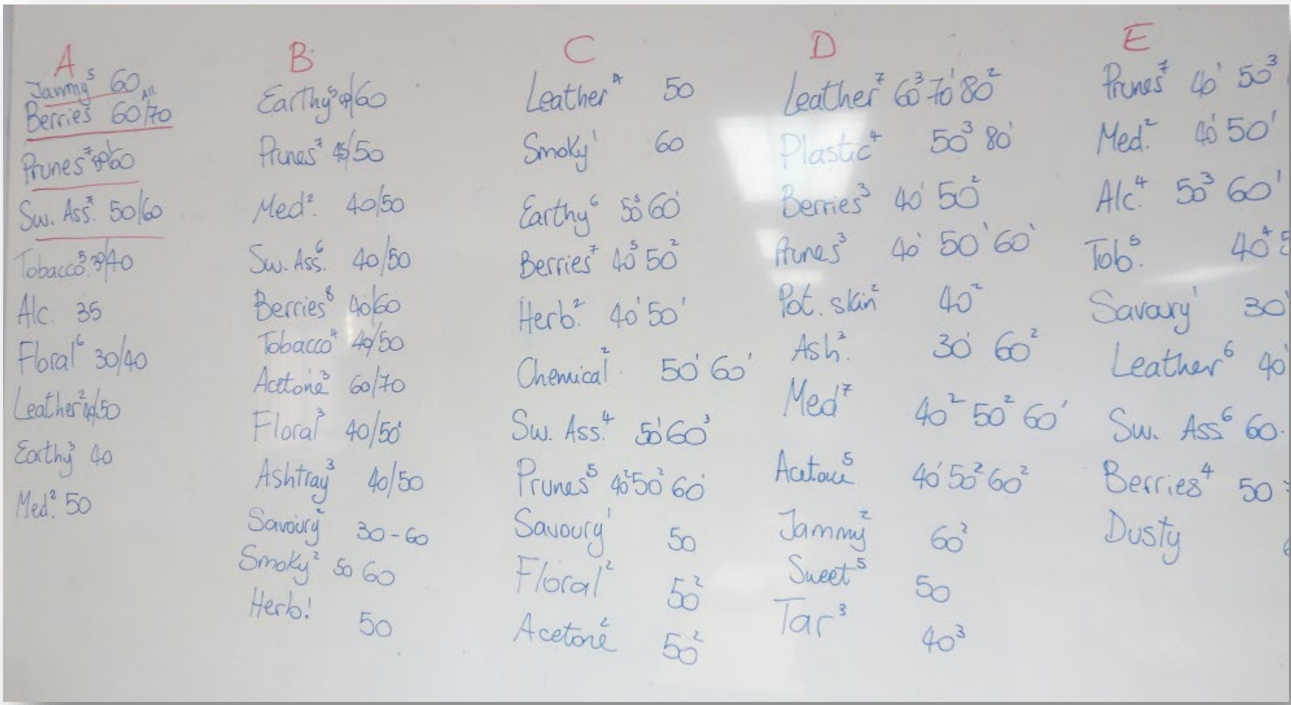
Wilson, D. & Stevenson, R., 2010. Learning to Smell. (2nd ed.). John Hopkins University Press.

Wilson, D.A., 2005. Odor Perception is Dynamic: Consequences for Interpretation of Odor Maps Chem. Senses 30, Supplement 1, i105–i106.

Wilson, D.A. & Sullivan, R.M., 2011. Cortical Processing of Odor Objects Neuron 72, 4, 506–519.

Yang, W., Li, W., *et al*., 2015. Odour prediction model using odour activity value from pharmaceutical industry Austrian Contrib. to Vet. Epidemiol. 8, 51–60.

Chapter 6



Investigation of olfactory interactions of low levels of five off-flavour causing compounds in a red wine matrix

This article is written in the style of the South African Journal of Enology and Viticulture to be submitted

Investigation of olfactory interactions of low levels of five off-flavour causing compounds in a red wine matrix

Marianne McKay [1*], Florian F. Bauer [2], Valeria Panzeri [3] and Astrid Buica[4]

[1]Department of Viticulture and Oenology; Stellenbosch University, marianne@sun.ac.za

[2]Department of Viticulture and Oenology; Stellenbosch University, fb2@sun.ac.za

[3]Institute for Grape and Wine Sciences; Stellenbosch University panzeri@sun.ac.za

[4]Department of Viticulture and Oenology; Stellenbosch University abuica@sun.ac.za

Key words: olfactory interactions; mixtures; volatile phenols; IBMP; TCA; DA;

Running title: *Sensory effects of binary mixtures of volatile phenols and IBMP in red wines*

Abstract

The qualitative sensory perception of individual and of complex mixtures of five compounds, guaiacol ('burnt note'), *o*-cresol ('phenolic/tar'), 4-EP ('leather/barnyard'), IBMP ('green pepper/herbaceous'), and TCA ('cork taint/ mouldy') were tested in a partially de-aromatised red wine matrix using descriptive analysis by a trained panel of eleven judges . Compounds were characterised at peri- and sub-threshold concentrations using a partial D-optimal statistical design and response surface methodology. Results indicated that complex mixtures in red wine elicit an olfactory response that could not be predicted from the attributes or descriptors of single compounds on their own. Positive sweet/fruity attributes were more intense in solutions containing fewer off-flavour compounds. Novel findings of this study include that IBMP at sub- and peri-threshold levels shows perceptual interaction with volatile phenols at the same levels, and samples containing combinations of these compounds manifested herbaceous and burnt characteristics. Olfactory interactions of so many off-flavour compounds have not been investigated previously in one study. The findings have direct implications for wines made from cultivars that are known to contain these compounds, and add to the understanding of the behaviour and impact of low levels of volatile phenols, IBMP and TCA derived from various sources during winemaking.

1. Introduction

Wine faults like microbial contamination and lack of typicality of style are often detected by olfaction (Perry & Hayes, 2016). Frequently used indicators of the potency of aroma compounds associated with aroma in wine and many other matrices include odour detection threshold (ODT) and odour activity value (OAV) measurements. In practice, a large number of issues influence the perception of the compounds involved, including matrix effects and the so-called 'sensory interaction' of compounds which affect their perception by judges.

Interactions between odorous components may impact the evaluation of sensory quality of food stuff. In wine, some olfactory antagonistic effects have been studied (Lapalus *et al.*, 2016; Tempere *et al.*, 2016; Wilson *et al.*, 2018) and several specific effects have been reported acetic acid and ethyl phenols mask fruity notes (Atanasova *et al.*, 2005; Campo *et al.*, 2005). In wine, volatile phenols are frequently reported as being present. These compounds can be derived from storage in oak barrels (Chatonnet *et al.*, 1992) which have undergone toasting/firing during production, or from smoke events near vineyards, and elevated levels can also be associated with the presence of *Brettanomyces* yeast infections (Curtin, et al., 2008; Botha, 2010). Although the attributes and odour detection thresholds of a number of VPs are discussed in the literature, the characteristics of specific VPs in combination with each other or other compounds are largely unexplored. Individually, guaiacol is known to impart a smoky, burnt character and exhibits the lowest aroma detection threshold of the VPs (Boidron *et al.*, 1988). Guaiacol is generally the most abundant of the VPs detected in smoke-affected wines (Kennison *et al.*, 2011; Ristic *et al.*, 2016), and is considered to be an important indicator for smoke-related taints. Although the impact of 'woodiness' on the perception of fruit odour in wine has been investigated (Atanasova *et al.*, 2005), there are no studies, to our knowledge, that combine volatile phenols with the classic 'green descriptor' IBMP or the compound largely responsible for 'cork taint' (TCA).

In Chapter 3 of this dissertation it has been shown that binary mixtures of volatile phenols and IBMP in red wine did not give olfactory results that can be predicted from the attributes of the single compounds in the matrix. Perception of odour mixtures containing more than two components is far more complicated due to interactions arising from the complex chemical signal encoding and processing within the olfactory system (Kaeppler & Mueller, 2013). In the case of complex mixtures, the odour quality of the mixture is more frequently different to the quality of odorants in the mixture, and it has been noted that complex mixtures are more likely to induce the perception of a new odour (Livermore & Laing, 1998; Ferreira, 2012).

The rationale for this study was to investigate whether a range of negative descriptors (for example, 'tar', 'burnt rubber', 'earthy', 'animal', 'dusty') that have on occasion been attributed to red wines can be explained by the interactions between volatile phenols (VPs), IBMP and TCA. One hypothesis to explain the perception of these descriptors is that the combination of such compounds could lead to the formation of new odour precepts/ odour objects as outlined by Thomas-Danguin *et al.,* (2014).

Previous research by Panzeri, (2013) showed olfactory perceptual effects when VPs were combined in red wine, in particular combinations involving 4-EP and *o*-cresol. In the current study, Descriptive Analysis (DA) was chosen to evaluate mixtures of volatile phenols and IBMP and TCA. DA is the most recognised sensory methodology, is quantitative, and can be used to describe differences between products and the main sensory drivers (whether positive or negative) (O'Sullivan & Byrne, 2011). Five odour-active compounds were selected that had been associated with specific taint issues in wine aroma (Table 1), namely guaiacol, *ortho*-cresol (*o*-cresol), 4-ethylphenol (4-EP), 3-isobutyl- 2-methoxypyrazine (IBMP) and 2,4,6-trichloranisole (TCA). Aroma descriptors for these compounds cover a continuum from 'burnt, smoky' and 'medicinal' to the 'herbaceous, green' aromas associated with IBMP.

Table 1. Odour Detection Thresholds (ODTs) in µg/L (unless otherwise indicated) in red wine and descriptors for compounds used in this study

Compound	ODT	Descriptors	Reference
guaiacol	23(red)	burnt, smoky, toasty, phenolic	Parker et al., (2012)
o-cresol	62 (red)	burnt, smoky, medicinal, tar	Parker et al., (2013)
4-EP	605 (red)	leather, bacon, medicinal, horse	Chatonnet et al., (1992)
IBMP	15 (red) ng/L	green, herbaceous, bell pepper	Roujou de Boubee et al., (2000)
TCA	3.7 ng/L*	mouldy, musty, damp cardboard	(Prescott et al., 2005)

* consumer rejection threshold

The aim of this study was to investigate the sensory effects of the five taint-related compounds listed above in a partially de-aromatised red wine matrix, in order to elucidate whether attributes associated with red wines, including 'burnt rubber', 'tar', and 'herbaceousness', might result from 'perceptual blending' and new odour-object formation resulting from complex mixtures of odorants. This has not been attempted with as many compounds in one sensory experiment previously.

2. Materials and Methods

2.1 Base wine

An unwooded 2016 Shiraz wine (300 L, pH 3.6, alcohol 13% v/v) was supplied by a local wine producer (Koelenhof Cellar Ltd, Simonsberg, South Africa) and stored at 4˚C in 25 L food-grade plastic containers under nitrogen at the Department of Viticulture and Oenology, Stellenbosch University, South Africa. Informal benchtop screening by five experienced sensory judges with tested sensitivity for the aroma compounds to be used in the study confirmed that the base wine was free of any form of 'mouldy' or 'herbaceous' odours that might have been associated with IBMP, and also any 'mouldy' or 'cork-taint' issues associated with TCA. The wine had an odour profile that was dominated strongly by fruit and berry aromas, which warranted partial de-aromatization with activated charcoal powder (Merck, Darmstadt, Germany) following the method outlined by (Wilson *et al.*, 2018) prior to the wine being used in investigations into sub-threshold olfactory interactions. In a screening session, the expert panel chose a blend of 50:50 charcoal-treated wine to untreated wine which yielded a neutral wine base with low aromatic intensity. Analysis of volatile phenols in the wine was performed by gas chromatography-massspectrometry (GC-MS) following the method outlined by De Vries, *et al.,* 2016 to determine the levels of the VPs present in the partially dearomatised base wine. The guaiacol level was 1.37 µg/L, *o*-cresol was 0.08 µg/L and 4-ethylphenol concentration was 1.4 µg/L.

2.2 Experimental design

As Descriptive Analysis is extremely time consuming, fatiguing and expensive and generates a large amount of complex data, a D-optimal statistical design was chosen before practical implementation. Wine samples were spiked with combinations of the five compounds at three levels each, following the partial D-optimal design (Table 2) constructed with Statistica 12.

Table 2. Spiking regime for 36 samples with five compounds. (Control sample shaded)

sample	D-Opt code	Guaiacol (µg/ L)	*o*-cresol (µg/ L)	4-EP (µg/L)	IBMP (ng/L)	TCA (ng/L)
1	0AB0B	0	62	400	0	2
2	A0AAA	23	0	605	10	4
3	00A00	0	0	605	0	0
4	BBAA0	15	40	605	10	0
5	BB000	15	40	0	0	0
6	0ABA0	0	62	400	10	0
7	B00AA	15	0	0	10	4
8	ABBB0	23	40	400	7	0
9	AAA00	23	62	605	0	0

Table 2 (cont.)

10	A0A00	23	0	605	0	0
11	0B0AB	0	40	0	10	2
12	A000B	23	0	0	0	2
13	AAAA0	23	62	605	10	0
14	00AA0	0	0	605	10	0
15	0BA0A	0	40	605	0	4
16	0AAAA	0	62	605	10	4
17	AB0BA	23	40	0	7	4
18	0AA00	0	62	605	0	0
19	AAA0A	23	62	605	0	4
20	BA0A0	15	62	0	10	0
21	AA00A	23	62	0	0	4
22	B0B0B	15	0	400	0	2
23	0A0AA	0	62	0	10	4
24	00000	0	0	0	0	0
25	0A00A	0	62	0	0	4
26	00ABA	0	0	605	7	4
27	AA0AB	23	62	0	10	2
28	BAABB	15	62	605	7	2
29	A00A0	23	0	0	10	0
30	000B0	0	0	0	7	0
31	A0A0A	23	0	605	0	4
32	0000A	0	0	0	0	4

Table 2 (cont.)

33	A0AAB	23	0	605	10	2
34	AABAA	23	62	400	10	4
35	AA000	23	62	0	0	0
36	00BAA	0	0	400	10	4

This design is ideal for multi-factor experiments with both quantitative and qualitative factors, at a mixed number of levels, as in this study. Combined with surface response methodology, this design generates contour plots by linear or quadratic effects of the key variables, and complex interactions can be visually represented. The D-optimal design is known to minimise the generalised variance of estimated regression coefficients (NCSS, 2010), and is useful when it is not possible to run a fully replicated factorial design, or there are budget and time constraints (like those associated with running a sensory panel).

2.3 Preparation of spiked samples

Stock solutions of 1000 mg/L of the four compounds were prepared in 99.5% ethanol (Merck Darmstadt, Germany). Guaiacol (99.3% purity), 4-EP (99.5% purity), *o*-cresol (99%), IBMP and TCA (also both 99%) were obtained from Merck, (Darmstadt, Germany). The compounds were dissolved in ethanol (10 mL) and then made up to volume with ultra-pure distilled water (Millipore, Bedford, MA, USA) to the concentrations required for spiking, i.e.: 100 mg/L for *o*-cresol and guaiacol; 1000 mg/L for 4-EP; and 5 µg/L for IBMP. Base wine was then spiked with an appropriate volume of stock solution to achieve the concentrations (Appendix 1, Figure A) of each volatile compound required for detection threshold determinations. These solutions were used to produce wine samples spiked with the desired concentrations of each compound. Using a 100 mg/L stock solution of guaiacol, 230 µL were added to each liter of de- aromatised red wine to achieve a 23 µg/L concentration in the sample. The stock solution of *o*-cresol was also 100 mg/L, so 620 µL was used per litre of wine to achieve the 62 µg/L detection threshold level. 4-EP was spiked with 0.605 mL of 1000 mg/L to reach 605 µg/L. The stock solution concentrations of IBMP and TCA were lower, and 3 mL, and 4 mL of each was used to achieve a final concentration of 10 ng/L, and 4 ng/L odour detection threshold levels for each compound respectively. For the subthreshold levels, a benchtop pre-screening established that 60-70% of the ODT did not add distinguishable attributes to the base wine. Base wine was spiked within 24 hours of sensory analysis and stored at 5˚C in the dark. Stock solutions were stored at 5˚C in brown, sealed glass bottles, with the exception of the IBMP stock solution which was stored at -20˚C in foil-wrapped containers to prevent light incursion. All levels used were subjected to extensive sensory pre-screening in order to determine whether they adhered to the sensory criteria set.

2.4 Panel Selection

The panel consisted of 11 judges, all non-smoking females between the ages of 24 and 60. Judges had previous experience in the use of quantitative descriptive analysis, and experience in smoke taint evaluation in wine. Most of the panellists also took part in the determination of odour detection thresholds for these compounds and therefore already had some familiarity with the compounds under investigation.

2.5 Sensory Training

A combination of consensus and ballot training was conducted before testing in six two-hour sessions over a period of two weeks. Twelve samples from the 36-sample D-optimal design (Table 2) as well as two clean controls were discussed in two consecutive sessions. Reference standards were presented in 50 mL amber glass bottles (Consol glass, RSA) following formulations adapted from Noble *et al*., (1987). For the first thirty minutes of each training session, panellists were asked to re-familiarise themselves with the specific aromas. The spiked wines were then profiled using descriptive analysis (DA) according to the general descriptive method (Lawless & Heymann, 2010). These discussions generated a comprehensive list of descriptors that included the familiar attributes, but also new attributes that were unique to the wines under study. The panel were also asked to rate the intensity of the various aromatic attributes. A final attribute list for testing was confirmed after the final training session. The twenty two attributes, agreed upon through consensus by the panel, included: dark berries, red berries, floral/ violets, prunes/raisins, vanilla/caramel, tobacco, pencil shavings, herbaceous/green; cooked veg., leather/barnyard, earthy/dusty/potato skin, smoky, ashtray, medicinal/Elastoplast, mouldy/musty, black pepper, liquorice, tar/ burnt rubber (BR), soy sauce, rubber/ chemical, acetone (nail varnish) and alcohol.

2.6 Sensory Testing

Sensory testing took place in six consecutive sessions over two weeks. Wines 1- 18 in the D-optimal design were tested in the first three sessions (in triplicate), with wines 19-36 replicated over the next three sessions. Although the panel was experienced in volatile phenol and smoke-taint related sensory analysis, in this project, 18 (3 flights of six) samples per testing session was considered the maximum that the panel would be able cope with without becoming fatigued. Testing was carried out in in a sensory laboratory equipped with individual booths with standard artificial daylight lighting and temperature control at 20 ± 1°C. Coded wines were presented to judges as eighteen samples in black ISO 3591:1977 standard glasses and covered with plastic lids. The order of samples was ‘counterbalanced’ across individuals, by changing the presentation order, as recommended by Lawless & Heymann (2010). All glasses were prepared one hour before serving to allow for temperature and headspace equilibration. Judges were asked to evaluate samples orthonasally (i.e., by sniffing). Communication was not allowed between the judges for the duration of the test.

2.7 Data analysis

Sensory data produced during Descriptive Analysis was analysed with Statistica, Version 12 (StatSoft, USA) to generate one-way and two-way ANOVAs, and Pareto charts of standardised effects combined with fitted Surface Response plots which were able to evaluate relative significance of several treatment factors in the presence of complex interactions. Least Squares Means (LSM) diagrams for attributes and compounds were produced from the two-way ANOVAS to determine significant differences between attributes and compounds, and to account for judge effects (different letters denoting significant differences at $p \leq 0.05$). Data were also examined for trends between attributes using Windows Excel (Microsoft Corp, Redmond, WA, USA). Principal Component Analysis (PCA) biplots were compiled from datasets for individual compound samples, and binary samples using PanelCheck to help explain variance (Lawless & Heymann, 2010). All quoted uncertainty is the standard deviation of three replicates of one treatment.

3. Results and Discussion

3.1 General overview of effects of interactions

The Principal Component Analysis (PCA) biplot generated from the sensory results of the individual compounds spiked at their ODTs (A) - and subthreshold (B) levels in partially dearomatised Shiraz wine (DSW) is shown in Figure 1. Attributes associated with five compounds (guaiacol (position 1), *o*-cresol (position 2), 4-EP (position 3), IBMP (position 4) and TCA (position 5) separate approximately according to fruity/ sweet-associated and earthy/herbaceous / burnt attributes, and the concentration of samples with a higher number of compounds can be seen on the biplot (yellow, orange and red colours).

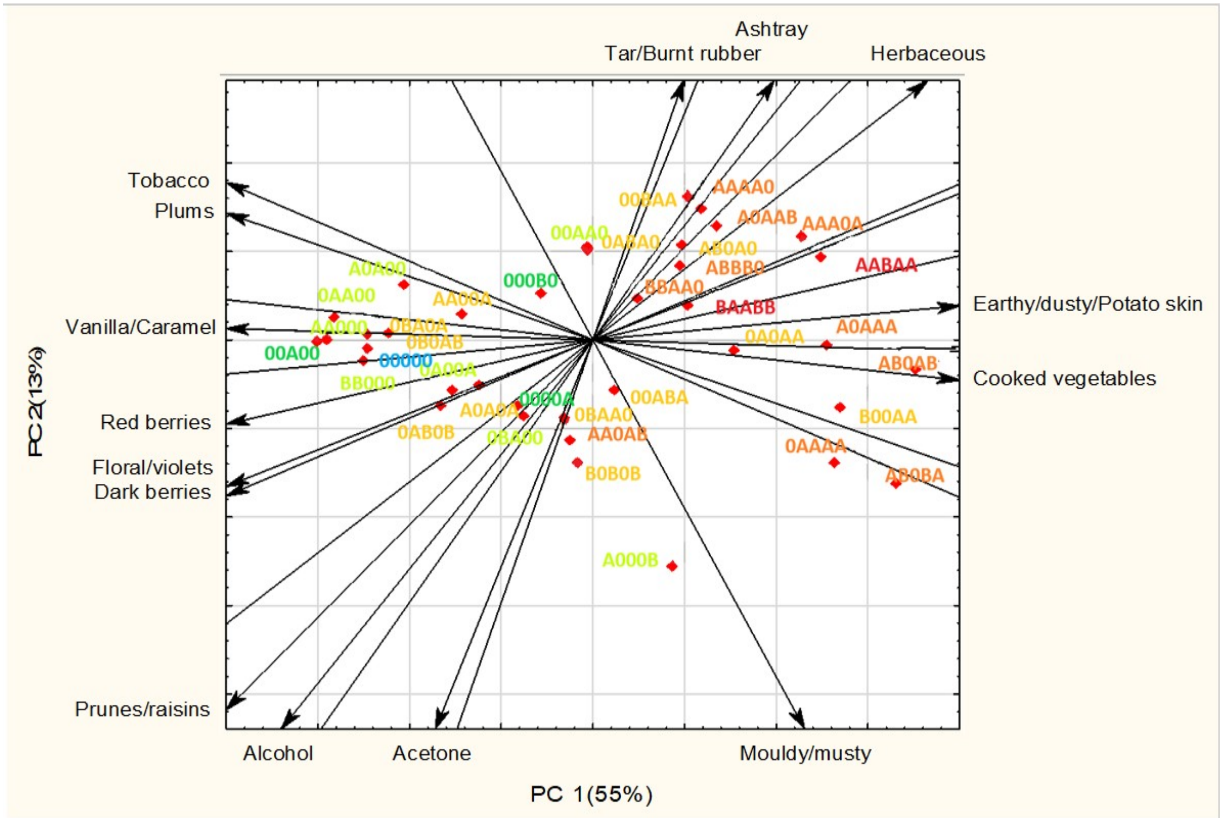


Figure 1. PCA biplot of the general sensory results of attributes of samples spiked with combinations of the three VPs, IBMP and TCA in dearomatised Shiraz wine in the following order: (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) and (position 5:TCA) at 0, peri- (A) and subthreshold (B) levels. Control sample (zero spike) in blue, single compound spikes in green, two compound spikes in light green, three compound spikes in gold, four compound spikes in orange and five compound spikes in red.

The first two principal components explain 68% of the variance in the data, which is considered satisfactory for sensory datasets. However, inspection of the PCA shows a tendency for more complex samples (> 3 components) to be associated with the burnt/chemical and earthy/ dusty negative attributes aligned on the positive side of PC1, and samples with fewer attributes (1-3 components- green and blue coloured) associated with the more positive sweet/fruity attributes on the negative side of PC1. It is notable that samples containing TCA (position 5) are not associated with the 'mouldy/musty' attribute, as is generally accepted to be the case. There does not seem to be a difference between peri- (A) and subthreshold (B) values on olfactory perception for the volatile phenols (first three positions in the sample code). There is an association between higher levels of IBMP and TCA with the 'earthy/dusty', 'cooked veg' and ashtray' attributes. Samples without IBMP and TCA do seem to position closer to the sweet-fruity 'tobacco', 'plums' and 'vanilla/caramel' attributes. The complexity of the PCA prompts an investigation of the sensory data to clarify effects of interactions of pairs of individual compounds.

3.2 Frequency of citation for five compounds

Attributes were subjected to a post-hoc examination of trends within the frequencies of citation. The frequency of citation (FC) of perceived attributes associated with control (clean) base wines (n = 108), wine spiked with single compounds (n =108), binary (n= 288), tertiary (n= 408), and quaternary mixtures (n=322) was assessed, as well as those associated with mixtures of all five compounds (n=72). Attributes were counted regardless of their intensity, and all attributes were allocated an equal weight in the frequency of citation, whether they were intense, or present at very low levels.

As can be seen from Figure 2, the FC of fruity/sweet-associated attributes generally decreased with increases in number of VPs or IBMP and TCA spiked into solution. This indicates that the panel's perception of the presence of these attributes gradually decreased as the mixture became more complex. This was independent of the nature of the attribute, and is consistent with the idea that a mixture of odorants could induce a note different from the one carried by its components as put forward by Barkat *et al* (2012). Positive 'sweet/fruity' attributes that are markedly affected by the presence of spiked compounds, and FC decreases as the complexity of the mixture increases include the 'prunes/raisins', both 'berry' attributes, 'plums', 'floral violets' and 'vanilla/caramel' attributes. Figure 2a shows that the 'tobacco' attribute is also cited less frequently in wine spiked with mixtures of volatile phenols, IBMP and TCA.

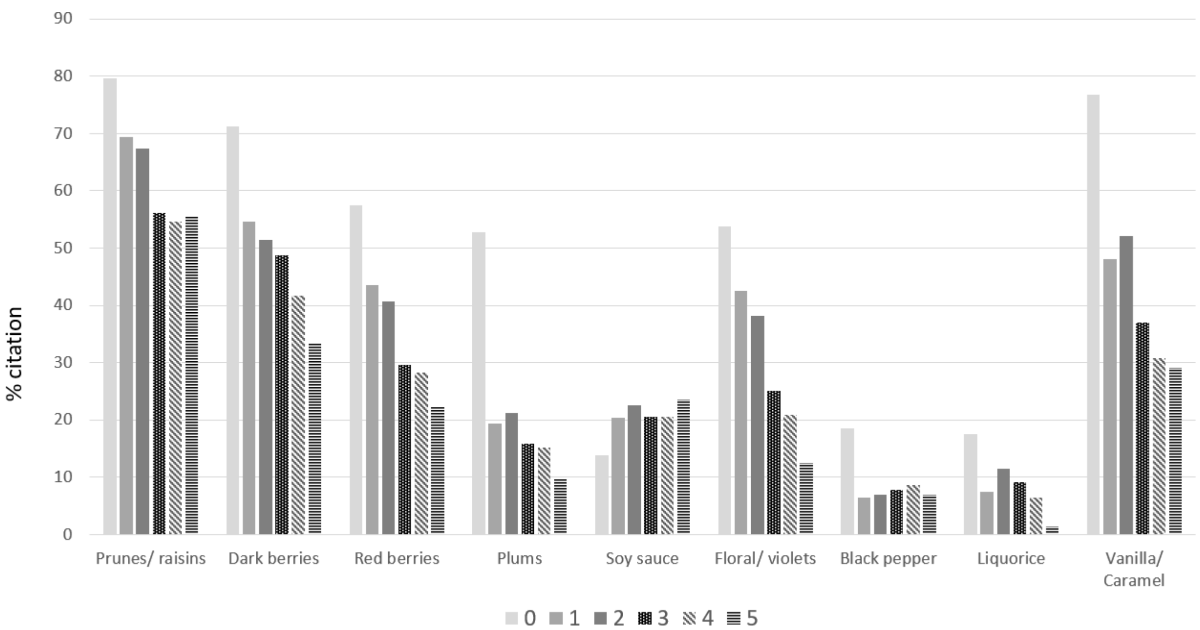
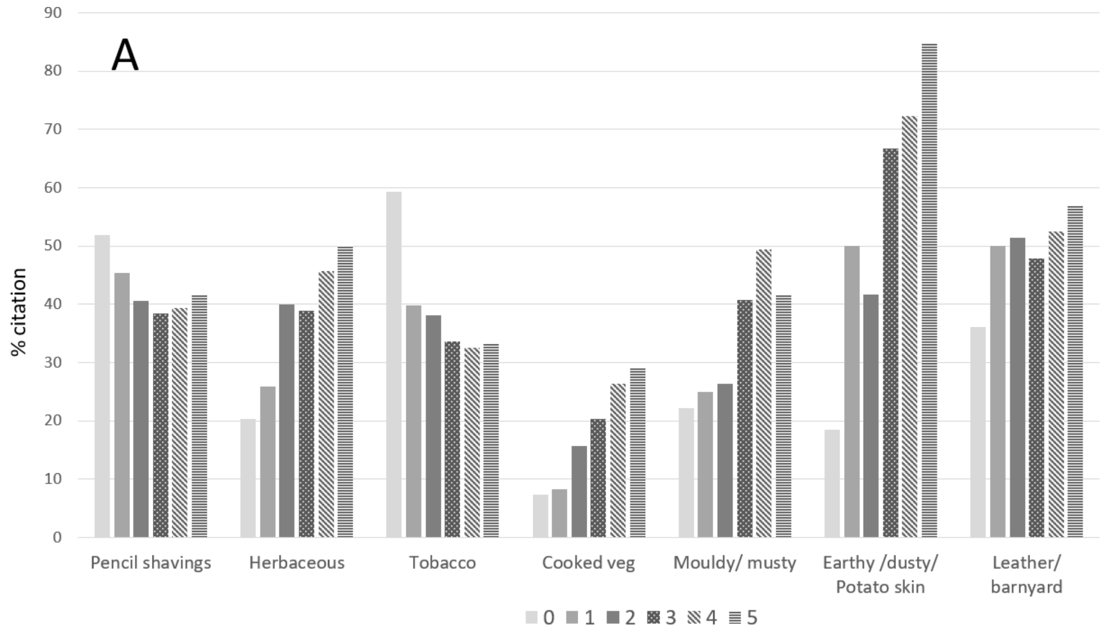


Figure 2. Effect of increasing number of spiked compounds (0-5) on frequency of citation (% of maximum possible counts of each attribute) of fruity / spicy attributes perceived in solution.

The FC of various attributes demonstrates that if mixtures were more complex, and contained greater numbers of volatile phenols, the fruity attributes were cited less frequently and attributes generally perceived as negative to wine quality, for example: green-associated attributes like 'herbaceous' and 'cooked veg', were perceived more often. This would be expected if IBMP was involved, but the FC is unrelated to whether samples did or did not contain IBMP. This trend is also seen in other attributes, specifically 'earthy/dusty/potato skin', 'mouldy/ musty', and to a lesser degree, 'leather/barnyard'.

Figure 3B illustrates the effects of increasing complexity of spiking regime on the ‘chemical’- and ‘burnt’- associated attributes. Here also, there seem to be some trends between perception of an attribute and increasing number of spiked compounds. The control sample (0) appears to have had some’ smoky’, ‘ashtray’ and’ tobacco’ attributes, but it is clear that the number of judges perceiving ‘tar/BR, ‘medicinal/Elastoplast, ‘rubber/chemical’ and ‘ashtray’ attributes are increased if more compounds are present. Wilson & Stevenson, (2010) noted that the more complex an odorant mixture is, and the more features of olfactory receptor activation overlap, the more difficult the task of perceptual grouping. It is not possible to gauge whether the panel would have created new odour-objects (as hypothesised in literature concerning olfactory perception) to describe the more complex solutions if they had been presented with these solutions for the first time, and were able to freely assign descriptors. It may be that the increases in frequency of citation are merely a representation of the ‘halo effect’ where attributes are assigned to the closest descriptor, losing some of the rich blending /interaction information, as suggested by (Barkat *et al.*, 2012) and that DA is not the ideal way to test for interaction effects.



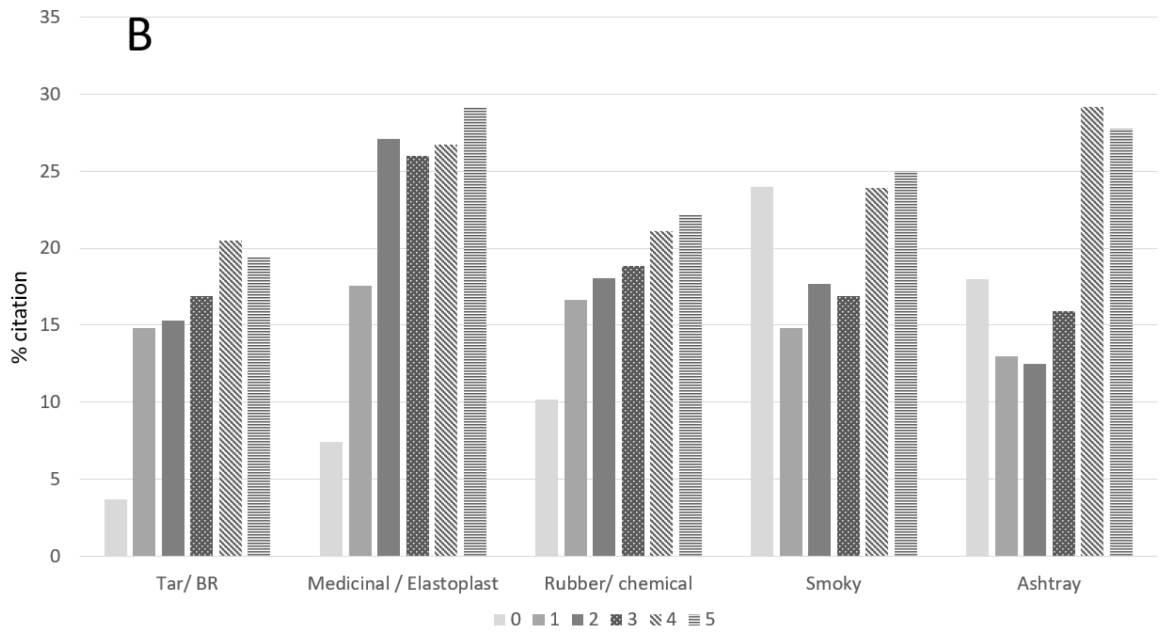


Figure 3. Effect of increasing number of spiked compounds (0-5) on frequency of citation (% of maximum possible count of each attributes) of A) woody and earthy attributes B) chemical/ burnt attributes perceived in solution.

3.3 Interaction effects between compounds

The information on significant effects per attribute was extracted from the Pareto chart of standardised effect estimates for each attribute (Table 2). Pareto charts plot ANOVA effect estimates, or standardised effect estimates in decreasing order of relative frequency sorted by size/ absolute value, with a vertical line to indicate the minimum magnitude of statistically significant effects, given the current model and choice of error term (TIBCO, 2017). Quadratic ('Q') effects denote statistical significance when the response surface contains curvature and the association is not just linear, 'L' denotes linear effect, and 'L by L' the linear interaction effects of pairs of variables/factors on the system. Changes in these factors are associated with changes in the response variable. If an interaction effect between two factors is significant, changes in each factor are associated with changes in the response variable, but the effects depend on the other factor. In this study, standardised effects estimates were illustrated, and p=0.05 was used as the criterion of statistical significance. Table 3 shows standardised effects for the study ($p < 0.05$) which estimate linear (L), quadratic (Q), and linear interactions (L by L) between factors. Although a great many smaller interactions and effects were observed as a result of the addition of the compounds to the de-aromatised red wine matrix in this study, only the most significant effects on perception of attributes will be discussed per compound, and then according to interactions between pairs of compounds.

Table 3: Standardised effects ($p<0.05$) per attribute extracted from Pareto's charts to estimate the linear (L), quadratic (Q), and linear interaction (L by L) between factors (Factor 1= guaiacol, 2= *o*-cresol, 3= 4-EP, 4= IBMP, 5= TCA) (ns= not significant at $p=0.05$)

Attribute	Pure error	Increased by	Standardised effect	Decreased by	Standardised effect
Dark berries	51.65	4 (Q)	4.02	3L by 4L	-2.81
		4L by 5L	2.59	4L by 4L	-2.37
		2L by 3L	2.04	1L by 5L	-2.28
		5 (Q)	2.02	2Q	-2.29
Red berries	40	5 (Q)	1.9	5L	-2.65
				4L	-2.4
Plums	20.81	5(Q)	2.01	1L	-2.01
				2L by 5L	-1.95
Floral / violets	27.8	5Q	3.13	5L	-3.54
		4Q	3.16	4L	-2.82
				3Lx4L	-2.60
Prunes raisins	38.6	5Q	2.13	4L	-4.36
		4Q	2.06		
		1L by 2L	2.01		
Vanilla caramel	40.85	5Q	3.35	4L	-4.08
		4Q	3.21		
		3Q	2.19		
Tobacco	26.22	4Q	2.59		
Pencil shavings	38.92			3L by 5L	-2.66

Table 3 (cont.)

Herbaceous	28.02	2L by 5L	3.12	4Q	-5.19
		3L by 4L	3.00	3Q	-3.75
		1L by 5L	2.77	4L by 5L	-3.41
		4L	2.29	2L by 3L	-2.43
		2L	1.96		
Cooked veg	21.64	4L	3.90	4Q	-4.80
		3L by 4L	3.33	3Q	-3.37
		2L by 4L	2.23	1Lby 2L	-3.14
		4L by 5L	1.95		
Leather / barnyard	42.5			3Q	-2.35
				4L by 5L	-2.14
Earthy/dusty/ potato	63	4L	4.92	5Q	-5.06
		5L	3.71	4Q	-4.95
		2L	1.75		
Smoky	27.8				
		3L by 4L	2.77	4Q	-3.3
		3L	2.39	1Q	-2.33
		5Q	1.96		
Ashtray	24.6	3L	2.82	4Q	-3.37
		2Q	2.5	1Q	-2.02
		1L by 3L	1.38 (ns)		
		2L by 5L	1.38 (ns)		

Table 3 (cont.)

Medicinal/Elastoplast	28.57	1L by 4L	2.43	1Q	-2.26
		1L by 3L	1.82 (ns)	2L by 4L	-2.24
				3L by 5L	-2.16
				5L	-1.99
Mouldy/ Musty	79.87	3L	2.48	5Q	-4.24
		4L	2.26	1L by 2L	-3.79
		2Q	2.19	3Q	-2.44
				2L by 5L	-1.76 (ns)
Tar/BR	25.42	3L by 4L	2.40	2L by 3L	-2.58
		1L by 4L	1.60 (ns)	4L by 5L	-2.34
Rubber/ chemical	21.75			3L by 4L	-2.32
				2L by 5L	-2.07

Soy sauce: no significant effects; Alcohol: no significant effects; Acetone: no significant effects

3.3.1 Effects per single compound

Three of the study attributes were not significantly affected by any of the five compounds at either level (sub- or peri-threshold), or any combination of compounds. These were 'soy sauce', 'alcohol' and 'acetone'.

Guaiacol on its own significantly decreased ($p<0.05$) perception of the attribute 'plums' as spiking levels increased across the linear range 0, 15 and 23 µg/L (Figure 2). The attributes 'smoky', 'ashtray' and 'medicinal/Elastoplast™' were decreased in a quadratic (non-linear) effect across increasing levels of guaiacol. The presence of guaiacol alone does not explain the increases in smokiness found with increasing complexity of added compounds, so clearly the other compounds had an effect on this descriptor, as will be seen in the ensuing discussion of interactions between compounds to produce various attributes. When *o*-cresol was present on its own in solution, the perception of 'dark berries' was decreased significantly relative to the control, 'mouldy/musty', 'herbaceous' and 'earthy/dusty/ potato skin' attributes were perceived to increase. This has not been shown previously in the limited number of studies that investigate the effects of this compound, where *o*-cresol has been associated with 'burnt' and 'phenolic' attributes (Table 1). It is interesting that sub- and peri-thresholds of *o*-cresol may be linked to herbaceousness and earthiness in red wine, even without the presence of pyrazines. 4-EP, the compound normally responsible for perception of 'medicinal/ Elastoplast™' and 'leather/ barnyard' attributes in previous studies (Chatonnet *et al.*, 1992; Romano *et al.*, 2009), did not have a significant effect on 'medicinal/Elastoplast™' in this experiment, and was perceived to decrease the 'leather/barnyard' attribute significantly, and the effect was quadratic. The attributes 'cooked veg', 'herbaceous' and 'mouldy/musty' attributes were also decreased by the addition of 4-EP on its own in solution. Perception of the 'smoky', 'ashtray' and 'vanilla/caramel' attributes were increased significantly compared to the control across the range of additions. The two non-phenolic compounds, IBMP and TCA, had quite complex effects as single compounds in solution on various attributes, sometimes exhibiting different results in their linear and quadratic conditions (Table 3).

IBMP had the most significant effect on its own in solution on the perception of a wide range of attributes in the study. Decreases (linear effect) were observed for sweet and fruity attributes, including 'floral/violets', 'red berries', 'prunes raisins', and 'vanilla/ caramel'. Increases in perception of 'herbaceous', 'cooked veg', 'earthy/ dusty/potato skin' and 'mouldy/musty' attributes were observed for increasing spike levels of IBMP. More subtle, quadratic effects of the single compound could been seen in its effect on the increases in perceptions of 'dark berries' and 'tobacco', and decreases in perception of 'smoky' and 'ashtray' attributes. TCA, reported frequently in the literature to be associated with 'cork taint' and 'mouldy/musty' aromas was seen, to decrease perception of 'earthy/dusty/potato skin' and 'mouldy/musty' in quadratic effect as a single compound in this study. Perception of the 'dark berry', 'floral / violets' and 'medicinal Elastoplast' attributes were decreased in linear effect, but a number of sweet /fruity attributes of the wine seemed to be affected positively ($p>0.05$) by this compound, which is counter-intuitive given its description as 'mouldy/ damp cardboard'. These included 'red berries', 'plums', 'prunes /raisins', 'vanilla/ caramel' and 'smoky'(Table 3).

3.3.2 Perceptual interactions of pairs of compounds

Guaiacol interaction with o-cresol (1L by 2L): Although not significant at the $p<0.05$ level, the 'ashtray' attribute showed an interesting trend to increase when these two phenols were in combination. The attribute 'prunes/raisins' was also significantly increased (Figure 5) by this interaction, which is also not explained by the descriptors for the compounds on their own in red wine. The attributes 'cooked veg' and 'mouldy musty' were significantly decreased ($p<0.05$) by the interaction effect of guaiacol and *o*-cresol.

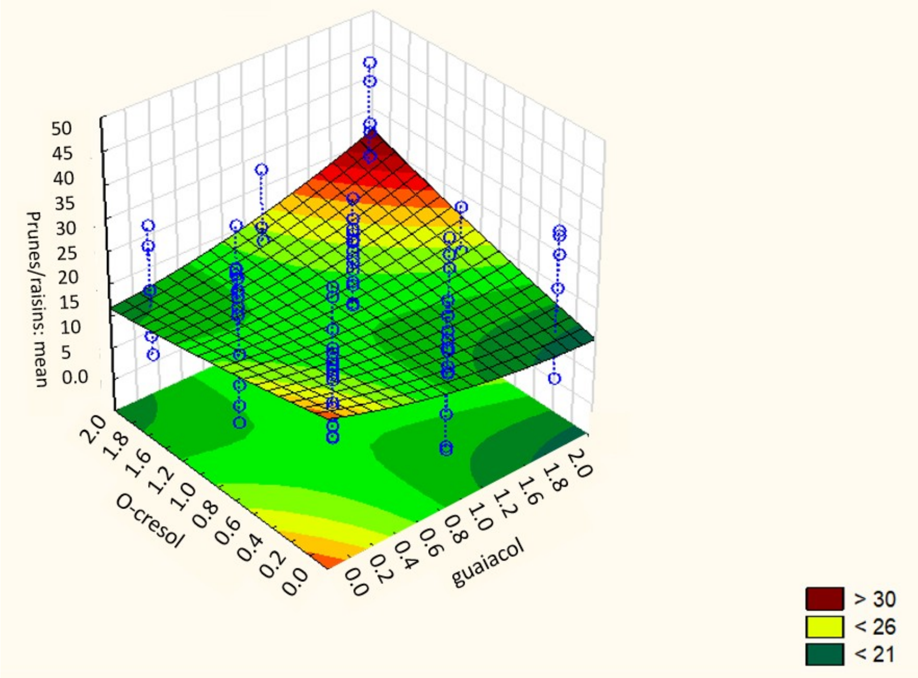


Figure 5. Fitted surface plot for 'prunes/ raisins' attribute means showing interactions between guaiacol and o- cresol (n=108)

Guaiacol interaction with 4-EP (1L by 3L): Again, the combination of two volatile phenols guaiacol and 4-EP led to a perception of the attribute 'ashtray' and 'medicinal/Elastoplast™' increasing, although this was not significant at the p=0.05 level.

Guaiacol interaction with IBMP (1L by 4L): Perception of the 'medicinal/Elastoplast™' attribute was significantly increased (p<0.05) by the interaction of guaiacol with IBMP. 'Tar/BR' was increased, as was 'leather / barnyard' (Figure 6) but these were not significant at p<0.05.

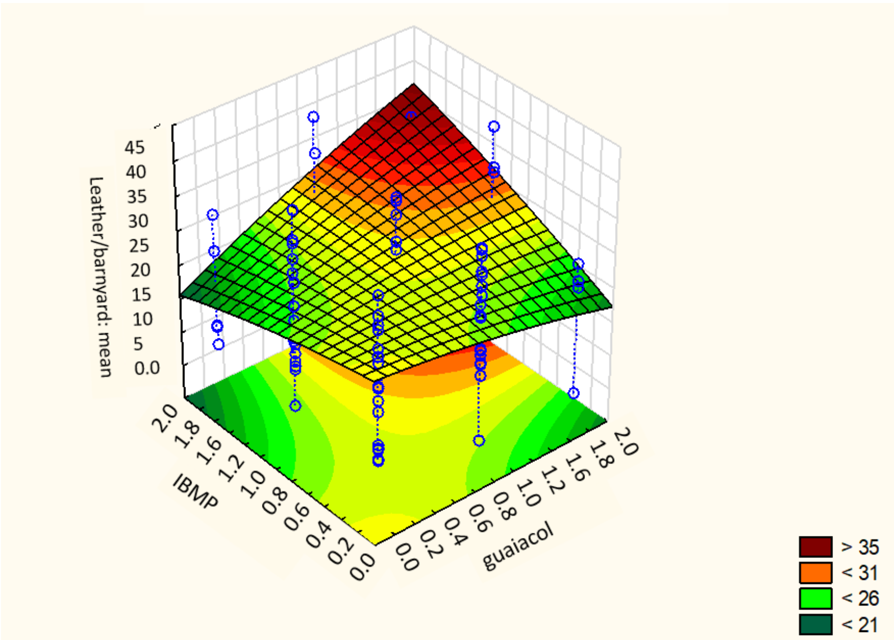


Figure 6. Fitted surface plot for 'leather/barnyard' attribute means showing interaction between guaiacol and IBMP (n=108)

Guaiacol interaction with TCAb(1L by 5L): The perception of the 'dark berries' attribute was decreased significantly (p<0.05) by the interaction of guaiacol with TCA compared to the control and single compound samples, but the perception of 'herbaceous' was increased significantly (Figure 7).

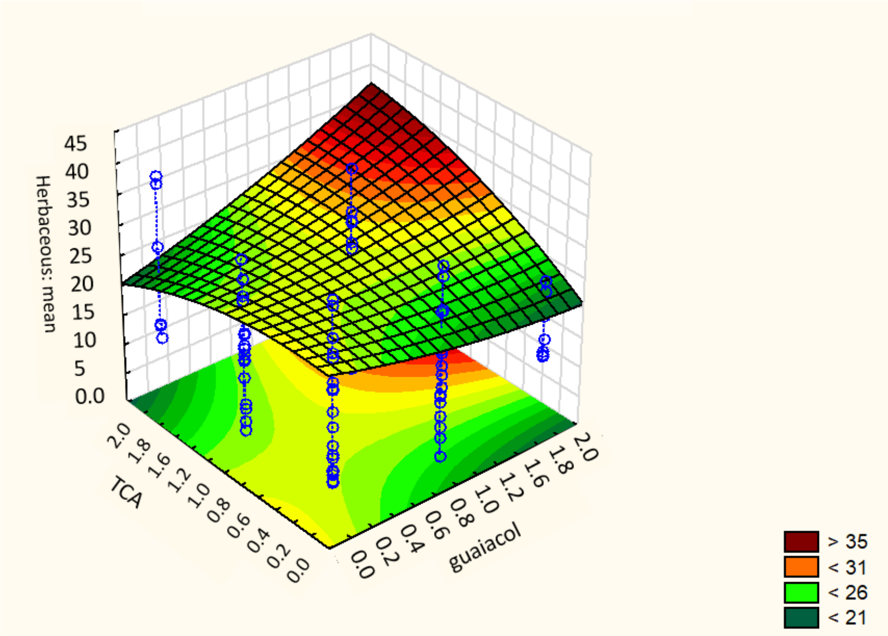


Figure 7. Fitted surface plot for 'herbaceous' attribute means showing interaction effect of guaiacol with TCA (n=108)

o-Cresol interaction with 4-EP (2L by 3L): The interaction of *o*-cresol with 4-EP lead to significant increases in perception (p<0.05) in 'leather/ barnyard', 'dark berry' and 'earthy/ dusty/potato skin (Figure 8)' attributes compared to control and single compound samples. The attributes 'herbaceous' and 'tar/BR' were perceived to decrease significantly, which is interesting given the findings of (Panzeri, 2013), that 4-EP in combination with other phenols caused increases in olfactory perception of this attribute. This may be due to matrix effects, which will be addressed in subsequent work.

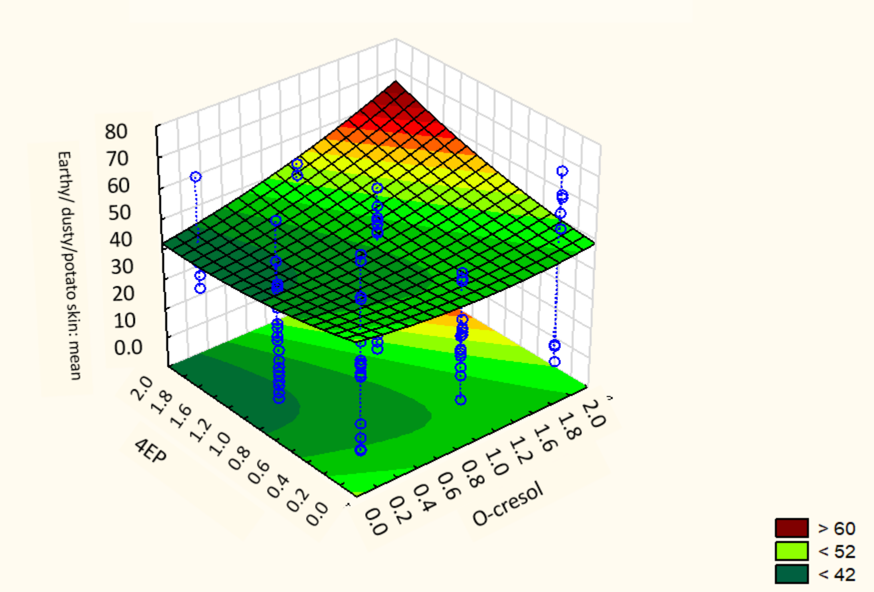


Figure 8. Fitted surface plot for Interaction effect of o-cresol with 4-EP on the 'earthy/dusty/potato skin' attribute (n=108)

o-Cresol interaction with IBMP (2L by 4L): Perception of the attributes 'cooked veg' and 'mouldy/ musty' (Figure 9) increased significantly ($p<0.05$) as a result of the interaction between IBMP and *o*-cresol. IBMP was shown to increase 'mouldy/musty' perception on its own, but it appears that the addition of *o*-cresol enhances the perception of this attribute. The perception of 'medicinal/Elastoplast™' was decreased by this interaction.

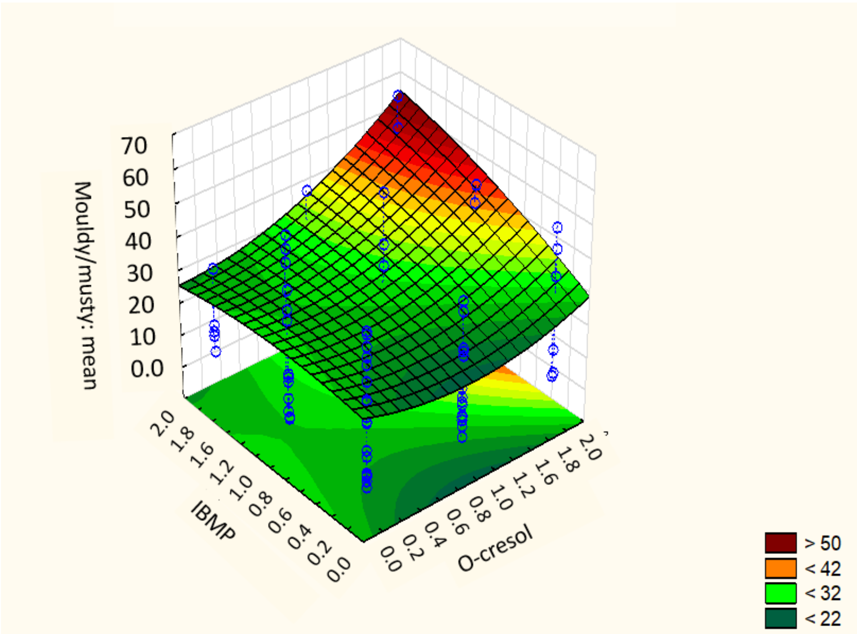


Figure 9. Fitted surface plot for Interaction effect of o-cresol with IBMP on the 'mouldy/musty' attribute (n=108)

o-Cresol interaction with TCA (2L x 5L):

The interaction of *o*-cresol and TCA causes an increase in the perception of 'herbaceous'($p<0.05$) and 'ashtray' (Figure 10) attributes and a decrease in the perception of 'plums', 'rubber/chemical and 'mouldy/musty' (all $p<0.05$).

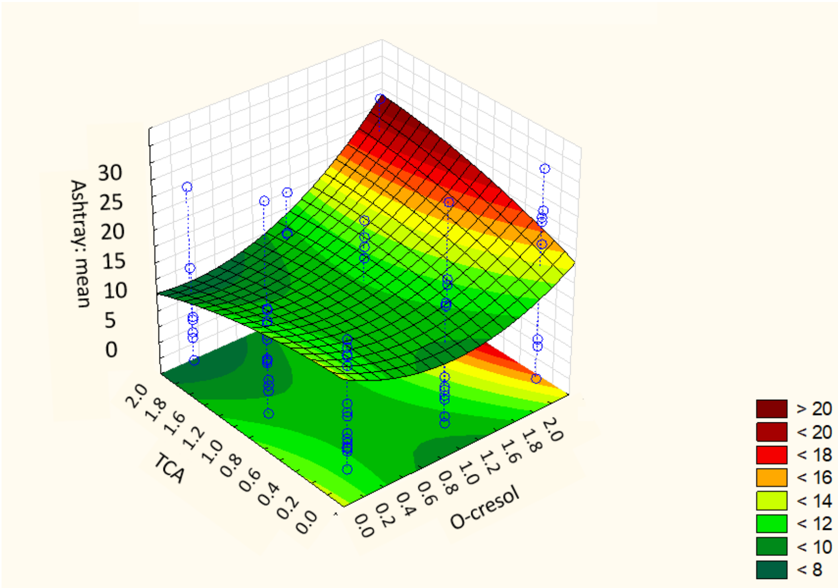


Figure 1. Fitted surface plot for interaction effect of o-cresol with TCA on the 'ashtray'/ attribute (n=108)

4-EP interaction with IBMP (3L x 4L): Perception of the attributes 'dark berries', 'rubber/chemical' and 'floral /violets' was decreased significantly ($p= 0.05$) by the interaction of IBMP and 4-EP. The attributes 'cooked veg', 'herbaceous', 'smoky' and 'tar/BR' (Figure 11) were seen to increase as a result of this interaction, which has implications for winemaking. In the event that cultivars (for example, Cabernet Sauvignon and Merlot Noir) are known to contain IBMP as part of their primary aroma profile (Allen *et al.*, 1996) producers should be alert to the fact that the presence of volatile phenols, even at low levels, may enhance herbaceous, cooked veg, smoky and tar/BR attributes, which may be perceived as off-flavours.

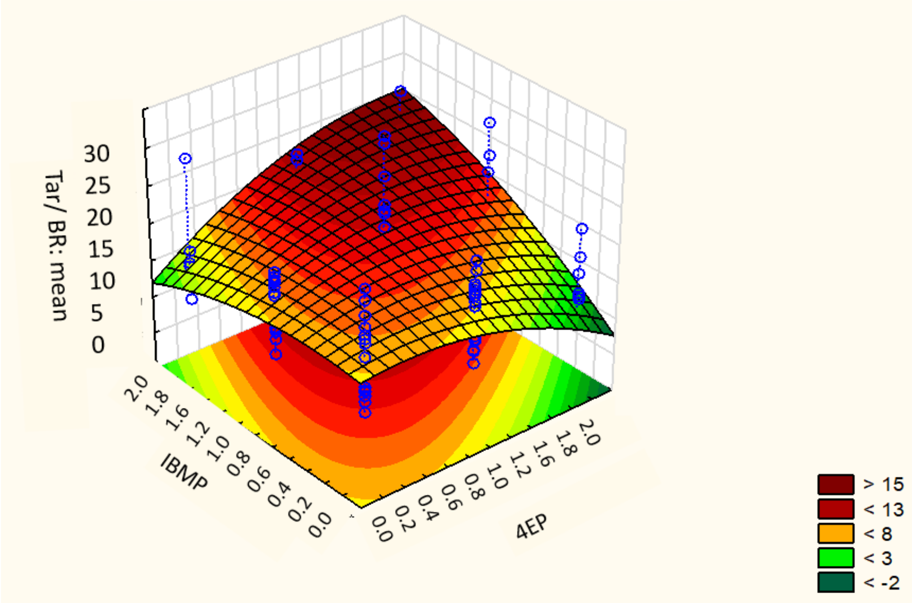


Figure 2. Fitted surface plot for Interaction effect of 4-EP with IBMP on the 'tar/BR' attribute (n=108)

4-EP interaction with TCA (3L x 5L): The interaction of 4-EP with TCA caused significant decreases in the perceptions of 'pencil shavings, and 'medicinal/Elastoplast™' compared to the original base wine

IBMP interaction with TCA (4L x 5L). The two non-phenolic compounds in the study showed some interesting interactions in combination. Unexpectedly, the perception of 'dark berries' was significantly increased ($p<0.05$), as was 'red berries (Figure 12). The 'herbaceous' 'leather /barnyard' and 'tar/BR' attributes decreased, but 'cooked veg' increased. These interactions indicate that perception between seemingly related attributes like 'herbaceous' and 'cooked veg' (both viewed as 'green') can be complex, and depend on additional factors such as matrix effects, fatigue and experience of the panel.

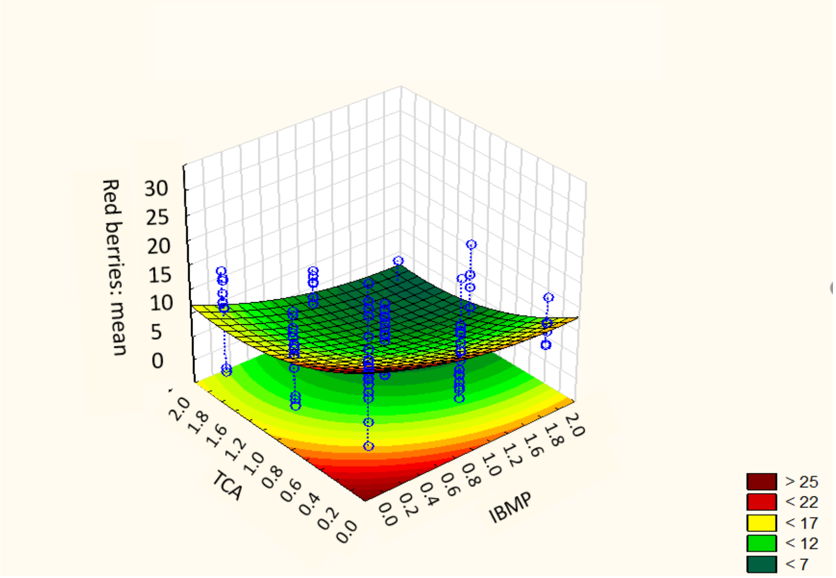


Figure 3. Fitted surface plot for Interaction effect of IBMP with TCA on the 'red berries' attribute (n=108)

3.2.3 Perceptual interactions between three or more compounds

A study of the Least Squares Means diagrams (Appendix 1: Figure B 1-15) for all the wines reveals that perceptions of increases in positive (fruity, sweet-associated) attributes, as previously noted in Section 3.2.2, are caused mainly by single or binary combinations of VPs, but decreases are associated with higher numbers of compounds in solution. The increase in perception of 'floral /violets' (Appendix 1: Figure B1) shows the caused by the combination of *o*-cresol and 4-EP in sample 0AA0), as well as the significant decreases compared to the control in samples containing IBMP and TCA (for example, 00ABA, 00BAA, 0B0AB). The 'prunes', 'red berries', 'vanilla/ caramel' and 'dark berries' attributes (Appendix1: Figure B2- B5) are also affected by combinations of samples containing VPs with IBMP and TCA, for example, 0B0AB, A0AAA, AB0BA and BAABB).

It is predominantly the earthy and chemical/ burnt related descriptors that seem to be increased in samples with three or more added compounds. The 'leather/barnyard' attribute (Appendix 1: Figure B6) is perceived to significantly increase over levels perceived in the control in the case of samples containing 4-EP at peri and subthreshold levels in combination with TCA and IBMP (samples 00BAA, 0BA0A and A0A0A). Even though a number of these perceptions were not significant, there are some notable trends, for example, the 'earthy/dusty/ potato skin' attribute (Figure 13), which shows increases compared to the control (sample 00000), in mixtures 0AAAA (peri-threshold levels of *o*-cresol, 4-EP, IBMP and TCA), 0B0AB (subthreshold levels of *o*-cresol and TCA, and peri-threshold levels of IBMP, AB0BA (peri-threshold levels of guaiacol and TCA, and subthreshold levels of *o*-cresol and IBMP. The 'cooked veg' attribute also shows some interesting increases in perception in combination compounds (Appendix 1, Figure B8), indicating that samples with VPs and both IBMP and TCA are affected (samples 0B0AB, A0AAA and AABAA, for example).

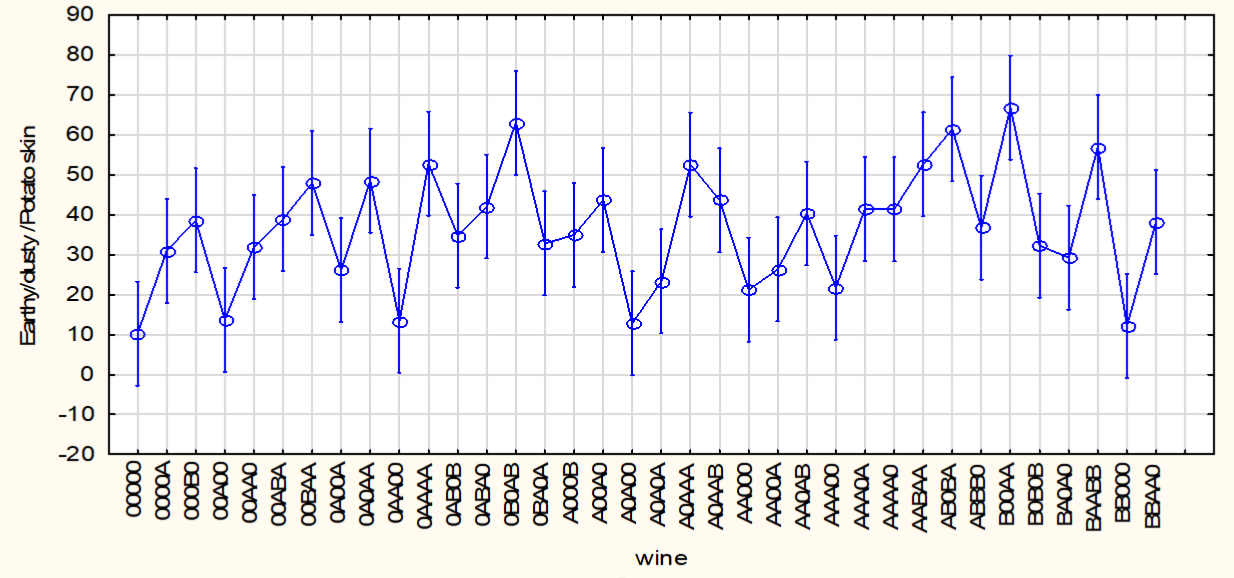


Figure 4. Least Squares Means (Type III decomposition) diagram for 'Earthy/dusty/potato skin' attribute (p=0.055). Sample codes denote spiking component and level: 0 denotes no spike, (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) and (position 5:TCA) at 0 peri (A) and subthreshold (B) levels. Vertical bars denote 0.95 confidence intervals.

The 'mouldy/ musty' attribute shows some interesting trends in samples containing combinations of TCA and VPs. When TCA was present as a single compounds, the effect on this attribute was not significant- indeed, TCA was seen to enhance some of the fruity aspects as a single compound. However, in combination with VPs, the 'mouldy/ musty' attribute is significantly increased (p<0.001) compared to the control (00000). This can be seen in Figure 14, particularly in samples 0AAAA, 0AB0B, 0BA0A, A0AAA, AB0BA, and B00AA.

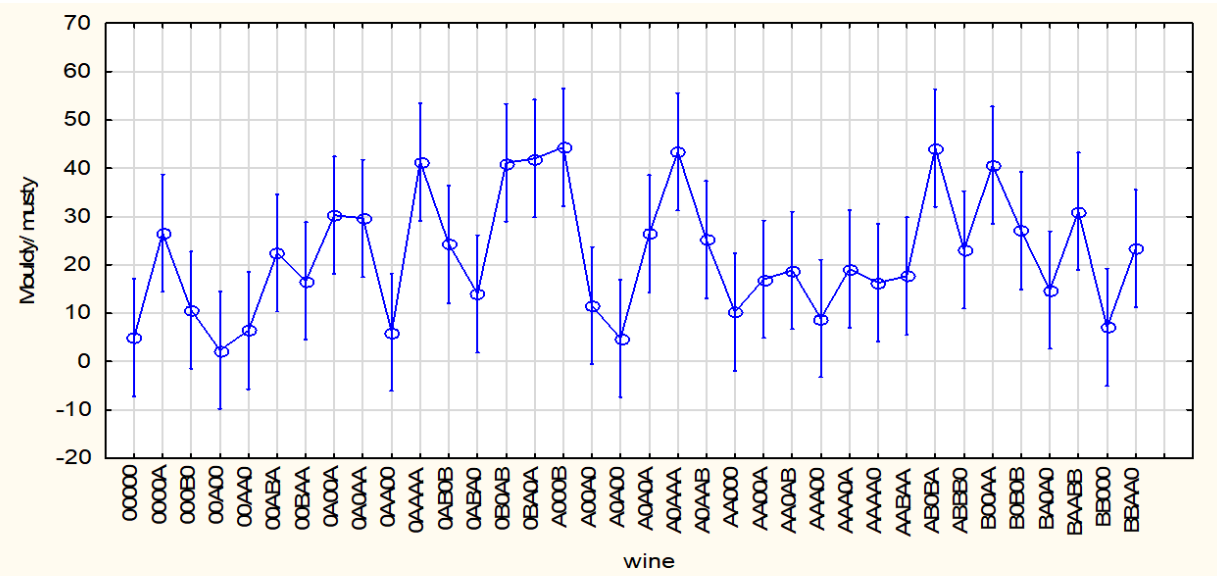


Figure 5. Least Squares Means (Type III decomposition) diagram for 'mouldy/musty' attribute (p<0.001). Sample codes denote spiking component and level: 0 denotes no spike, (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) and (position 5:TCA) at 0 peri (A) and subthreshold (B) levels. Vertical bars denote 0.95 confidence intervals.

Although the attribute was present at a low level, the impact of combinations of compounds can also be seen in the LSM diagram for the 'ashy/ashtray' attribute (Figure 15). When *o*-cresol or guaiacol are present in combination with TCA and/or IBMP, this characteristic seems to be enhanced. The 'smoky' attribute is enhanced when 4-EP is in combination with IBMP (Appendix 1, Figure B13). This enhancement also affects the perception of the 'herbaceous' attribute (increased when IBMP or TCA are in combination with volatile phenols, as in samples 0AAAA, A0AAA, AABAA, 0BA0A and AAAA0).

This trend also affects the attribute 'tar/BR' which is likewise increased (not significantly) when volatile phenols and either TCA or IBMP are present, as in sample 00BAA, AAAA0 and A0AAB.

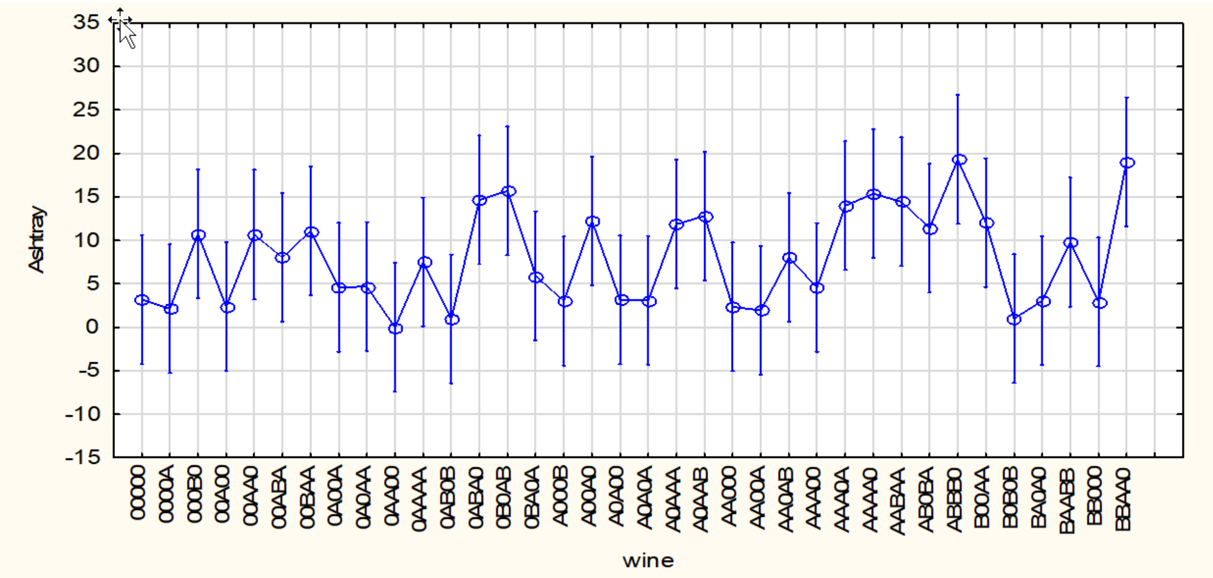


Figure 6. Least Squares Means (Type III decomposition) diagram for 'Ashy' attribute (p<0.001). Sample codes denote spiking component and level: 0 denotes no spike, (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) and (position 5:TCA) at 0 peri (A) and subthreshold (B) levels. Vertical bars denote 0.95 confidence intervals.

4. Conclusions

In order to explore interactions between five taint compounds (guaiacol, *o*-cresol, 4-EP, TCA and IBMP) in red wine, base wine was spiked with 2 different levels (per-threshold and subthreshold) for each compound in a partial D-optimal design. Specific interactions between pairs of compounds led to unexpected effects on perception, as was also noted in Chapter 3 *(Perceptual interaction and characterisation of odour quality of binary mixtures of volatile phenols and 2-isobutyl-3-methoxypyrazine in a red wine matrix)*. Results showed that combinations of three or more compounds in solution also led to unexpected effects in olfactory perception. Examination of the results indicated that for a number of attributes, frequency of citation in mixtures decreases (including 'plums', 'prunes', 'dark berries' 'red berries', 'floral/violet, 'vanilla/caramel' and 'tobacco'). These are generally considered positive sweet/fruity attributes and this indicates that combinations of volatile phenols, IBMP and TCA in wine leads to the reduction in fruitiness and perceived quality due to interaction effects even if these compounds are present at very low levels.

The PCA of general sensory results, as well as LSM diagrams of combinations of compounds, also show that samples with less complex composition (zero to three components) were associated with 'plums', 'vanilla/caramel', 'red berries', 'floral/ violet' and 'dark berry' attributes. Samples with a more complex structure, and n>3 off-flavour components spiked at peri-threshold levels were associated with 'cooked veg', 'earthy/dusty/potato skin', 'herbaceous', 'ashtray' and 'tar/BR' attributes on the biplot. In the frequency of citation study, the attributes 'earthy/ dusty/potato skin', 'mouldy/musty', 'herbaceous', 'ashtray', 'tar/BR', 'rubber/chemical' and 'medicinal/Elastoplast™' were increased as number of compounds increased in solution. These generally negative attributes were not strongly associated with the descriptors assigned by the panel to the solutions containing single components, and did not always agree with expectations created by previous studies. TCA at low levels in combination with VPs and IBMP increased the perception of the 'mouldy/musty' attribute which did not happen when the compound was present on its own in red wine. In samples containing IBMP, the attributes 'cooked veg', 'herbaceous', 'smoky' and 'tar/BR' increased as a result of interactions with this compound at sub- and peri-threshold levels, which has implications for winemaking. Red cultivars (for example, Cabernet Sauvignon and Merlot Noir) that are known to contain IBMP as part of aroma profile the presence of volatile phenols, even at low levels, may manifest green characteristics such as herbaceousness and grassiness even if IBMP levels are very low. It would be interesting to test whether sub- and peri-threshold levels of volatile phenols and other off-flavour compounds induce similar or very different characteristics in different wine matrices, and future work should include cultivars that have naturally higher levels of IBMP.

This study demonstrated perceptual effects of five off-flavour compounds in a red wine matrix, which have not been previously characterised, and showed clearly that negative characteristics may be induced or enhanced by their interaction. This work also showed that positive characteristics in wine like fruity and sweet attributes are reduced significantly as a result of interactions of low levels of phenols, IBMP and TCA. In future work, a sensory strategy other than DA may lead to richer results and different descriptors for complex solutions.

Acknowledgments: Winetech and THRIP (NRF) acknowledged funding associated with volatile phenol projects. Martin Kidd carried out the statistical analysis.

Author Contributions: Marianne McKay, Astrid Buica and Florian Bauer conceived and designed this study; Marianne McKay carried out all the practical work for the project. Valeria Panzeri led the sensory panel; Marianne McKay wrote the paper, which was revised and edited by Astrid Buica, Florian Bauer, and Valeria Panzeri.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

Allen, M.S., Lacey, M.J., *et al*., 1996. Existence of different origins for Methoxypyrazines of grapes and wines In:

G.R. Takeoka, R. Teranishi, P.J. Williams, & A. Kobayashi (eds). Biotechnol. Improv. Foods Flavors. American Chemical Society, Washington DC 220–227.

Atanasova, B., Thomas-Danguin, T., *et al*., 2005. Perceptual interactions in odour mixtures: odour quality in binary mixtures of woody and fruity wine odorants. Chem. Senses 30, 3, 209–17.

Barkat, S., Le Berre, E., *et al*., 2012. Perceptual Blending in Odor Mixtures Depends on the Nature of Odorants and Human Olfactory Expertise Chem. Senses 37, 2, 159–166.

Boidron, J.N., Chatonnet, P., *et al*., 1988. Influence du bois sur certaines substances odorantes des vins Connaiss. la vigne du vin 22, 4, 275–294.

Botha, J.J., 2010. Sensory, chemical and consumer analysis of Brettanomyces spoilage in South African wines Stellenbosch University.

Campo, E., Ferreira, V., *et al*., 2005. Prediction of the Wine Sensory Properties Related to Grape Variety from Dynamic-Headspace Gas Chromatography−Olfactometry Data J. Agric. Food Chem. 53, 14, 5682–5690.

Chatonnet, P., Dubourdie, D., *et al*., 1992. The origin of Ethylphenols in wines J. Sci. Food Agric. 60, 2, 165–178.

Curtin, C.; Bramley, B., Cowey, G., Holdstock, M., Lattey, K., Coulter, A., Henschke, P. Francis, L. Godden, P., 2008. Sensory perception of Brett and relationship to consumer preference In: I.. Blair, R.J and Pretorius (ed). Proc. 13th Aust. Wine Ind. Tech. Conf. Australian Wine Industry Techn. conf., Adelaide 207–211.

Ferreira, V., 2012. Revisiting psychophysical work on the quantitative and qualitative odour properties of simple odour mixtures: a flavour chemistry view. Part 2: qualitative aspects. A review. Flavour Frag. J. 27, 3, 201– 215.

Kaeppler, K. & Mueller, F., 2013. Odor Classification: A Review of Factors Influencing Perception-Based Odor Arrangements Chem. Senses 38, 3, 189–209.

Kennison, K.R., Wilkinson, K.L., *et al*., 2011. Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties Aust. J. Grape Wine Res. 17, 2, 5–12.

Lapalus, E., Wessel, P., *et al*., 2016. Linking sensory attributes to selected aroma compounds in South African Cabernet Sauvignon wines Stellenbosch University.

Lawless, H.T. & Heymann, H., 2010. Sensory Evaluation of Foods: Principles & Practices. (2nd ed.). Springer Science Business Media LLC, New York.

Lawless, H. & Heymann, H., 2010. Measurement of Sensory Thresholds. (2nd Ed..). Springer Science & Business Media (Food Science Text Series), New York.

Livermore, A. & Laing, D.G., 1998. The influence of odor type on the discrimination and identification of odorants in multicomponent odor mixtures. Physiol. Behav. 65, 2, 311–20.

O'Sullivan, M.G. & Byrne, D.V., 2011. Use of sensory science as a practical commercial tool in the development of consumer-led processed meat products Process. Meats (January, 1), 156–182.

Panzeri, V., 2013. Influence of vineyard posts type on the chemical and sensorial composition of Sauvignon blanc and Merlot Noir wines.

Parker, B.M., Baldock, G., *et al*., 2013. Seeing through smoke Wine Vitic. J. 42–46.

Parker, M., Osidacz, P., *et al*., 2012. Contribution of Several Volatile Phenols and Their Glycoconjugates to Smoke- Related Sensory Properties of Red Wine J. Agric. Food Chem. 60, 10, 2629–2637.

Perry, D.M. & Hayes, J.E., 2016. Effects of matrix composition on detection threshold estimates for Methyl Anthranilate and 2-Aminoacetophenone Foods 5, 2, 35–45.

Prescott, J., Norris, L., *et al*., 2005. Estimating a "consumer rejection threshold" for cork taint in white wine Food Qual. Prefer. 16, 4, 345–349.

Ristic, R., Fudge, A.L., *et al*., 2016. Impact of grapevine exposure to smoke on vine physiology and the composition and sensory properties of wine Theor. Exp. Plant Physiol. 28, 1, 67–83.

Romano, A., Perello, M.C., *et al*., 2009. Sensory and analytical re-evaluation of “Brett character” Food Chem. 114, 1, 15–19.

Tempere, S., Schaaper, M.H., *et al*., 2016. The olfactory masking effect of ethylphenols: Characterization and elucidation of its origin Food Qual. Prefer. 50, 135–144.

Thomas-Danguin, T., Sinding, C., *et al*., 2014. The perception of odor objects in everyday life: a review on the processing of odor mixtures Front. Psychol. 5, June, 1–18.

TIBCO, S.I., 2017. STATISTICA Help, Analysis of a Central Composite (Response Surface) Experiment

Available at http://documentation.statsoft.com/STATISTICAHelp.aspx?path= Experimental/Doe/Dialogs/CentralComposite/AnalysisofaCentralCompositeResponseSurfaceExperimentANO VAEffectsTab

Wilson, C., Brand, J., *et al*., 2018. Interaction Effects of 3-Mercaptohexan-1-ol (3MH), Linalool and Ethyl Hexanoate on the Aromatic Profile of South African Dry Chenin blanc Wine by Descriptive Analysis (DA) South African J. Enol. Vitic. 39, 2, 271–283.

Wilson, D. & Stevenson, R., 2010. Learning to Smell. (2nd ed.). John Hopkins University Press.

Appendix 1: Supplemental data

Table A: Spiking regime for partial D-optimal design for 250ml dearomatised red wine

	Sample	compound	level	spike
1	0AB0B	*o*-cresol	62ug/L	160ul x 100ppm
		4-EP	400ug/L	100ul x 1000ppm
		TCA	2ng/L	0.5ml x 1pg/ul
2	A0AAA	guaiacol	23ug/L	60ul x 100ppm
		4-EP	600ug/L	150ul x1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	4ng/L	1ml x 1pg/ul
3	00A00	4-EP	600ug/L	150ul x1000ppm
4	BBAA0	guaiacol	15ug/L	40ulx 100ppm
		o-cresol	40ug/L	100x 100ppm
		4-EP	600ug/L	150ul x1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
5	BB000	guaiacol	15ug/L	40ulx 100ppm
		o-cresol	40ug/L	100x 100ppm
6	0ABA0	*o*-cresol	62ug/L	160ul x 100ppm
		4-EP	400ug/L	100ul x 1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
7	B00AA	guaiacol	15ug/L	40ulx 100ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	4ng/L	1ml x 1pg/ul
8	ABBB0	guaiacol	23ug/L	60ul x 100ppm
		o-cresol	40ug/L	100x 100ppm
		4-EP	400ug/L	100ul x 1000ppm
		IBMP	7ng/L	0.36ml x 5ug/L
9	AAA00	guaiacol	23ug/L	60ul x 100ppm
		o-cresol	62ug/L	160ul x 100ppm
		4-EP	600ug/L	150ul x1000ppm
10	A0A00	guaiacol	23ug/L	60ul x 100ppm
		4-EP	600ug/L	150ul x1000ppm
11	0B0AB	*o*-cresol	40ug/L	100x 100ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	2ng/L	0.5ml x 1pg/ul
12	A000B	guaiacol	23ug/L	60ul x 100ppm
		TCA	2ng/L	0.5ml x 1pg/ul
13	AAAA0	guaiacol	23ug/L	60ul x 100ppm
		o-cresol	62ug/L	160ul x 100ppm

		4-EP	600ug/L	150ul x1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
14	00AA0	4-EP	600ug/L	150ul x1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
15	0BA0A	*o*-cresol	40ug/L	100x 100ppm
		4-EP	600ug/L	150ul x1000ppm
		TCA	4ng/L	1ml x 1pg/ul
16	0AAAA	*o*-cresol	62ug/L	160ul x 100ppm
		4-EP	600ug/L	150ul x1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	4ng/L	1ml x 1pg/ul
17	AB0BA	guaiacol	23ug/L	60ul x 100ppm
		o-cresol	40ug/L	100x 100ppm
		IBMP	7ng/L	0.36ml x 5ug/L
		TCA	4ng/L	1ml x 1pg/ul
18	0AA00	*o*-cresol	62ug/L	160ul x 100ppm
		4-EP	600ug/L	150ul x1000ppm
19	AAA0A	guaiacol	23ug/L	60ul x 100ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	4ng/L	1ml x 1pg/ul
20	BA0A0	guaiacol	15ug/L	40ulx 100ppm
		o-cresol	62ug/L	160ul x 100ppm
		IBMP	10ng/L	0.5ml x 5ug/L
21	AA00A	guaiacol	23ug/L	60ul x 100ppm
		o-cresol	62ug/L	160ul x 100ppm
		TCA	4ng/L	1ml x 1pg/ul
22	B0B0B	guaiacol	15ug/L	40ulx 100ppm
		4-EP	400ug/L	100ul x 1000ppm
		TCA	2ng/L	0.5ml x 1pg/ul
23	0A0AA	*o*-cresol	62ug/L	160ul x 100ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	4ng/L	1ml x 1pg/ul
24	00000	**(control)**		
25	0A00A	*o*-cresol	62ug/L	160ul x 100ppm
		TCA	4ng/L	1ml x 1pg/ul
26	00ABA	4-EP	600ug/L	150ul x1000ppm
		IBMP	7ng/L	0.36ml x 5ug/L
		TCA	4ng/L	1ml x 1pg/ul
27	AA0AB	guaiacol	23ug/L	60ul x 100ppm
		o-cresol	62ug/L	160ul x 100ppm
		IBMP	10ng/L	0.5ml x 5ug/L

		TCA	2ng/L	0.5ml x 1pg/ul
28	BAABB	guaiacol	15ug/L	40ulx 100ppm
		o-cresol	62ug/L	160ul x 100ppm
		4-EP	600ug/L	150ul x1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	2ng/L	0.5ml x 1pg/ul
29	A00A0	guaiacol	23ug/L	60ul x 100ppm
		IBMP	10ng/L	0.5ml x 5ug/L
30	000B0	IBMP	7ng/L	0.36ml x 5ug/L
31	A0A0A	guaiacol	23ug/L	60ul x 100ppm
		4-EP	600ug/L	150ul x1000ppm
		TCA	4ng/L	1ml x 1pg/ul
32	0000A	TCA	4ng/L	1ml x 1pg/ul
33	A0AAB	guaiacol	23ug/L	60ul x 100ppm
		4-EP	600ug/L	150ul x1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	2ng/L	0.5ml x 1pg/ul
34	AABAA	guaiacol	23ug/L	60ul x 100ppm
		o-cresol	62ug/L	160ul x 100ppm
		4-EP	400ug/L	100ul x 1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	4ng/L	1ml x 1pg/ul
35	AA000	guaiacol	23ug/L	60ul x 100ppm
		o-cresol	62ug/L	160ul x 100ppm
36	00BAA	4-EP	400ug/L	100ul x 1000ppm
		IBMP	10ng/L	0.5ml x 5ug/L
		TCA	4ng/L	1ml x 1pg/ul

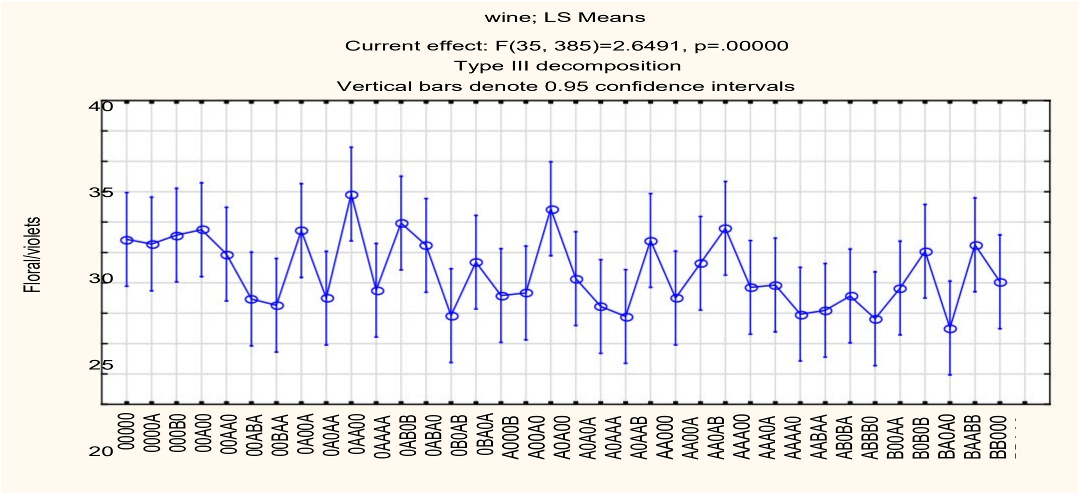


Figure B1: Least squares means diagrams for attributes: Floral/violets

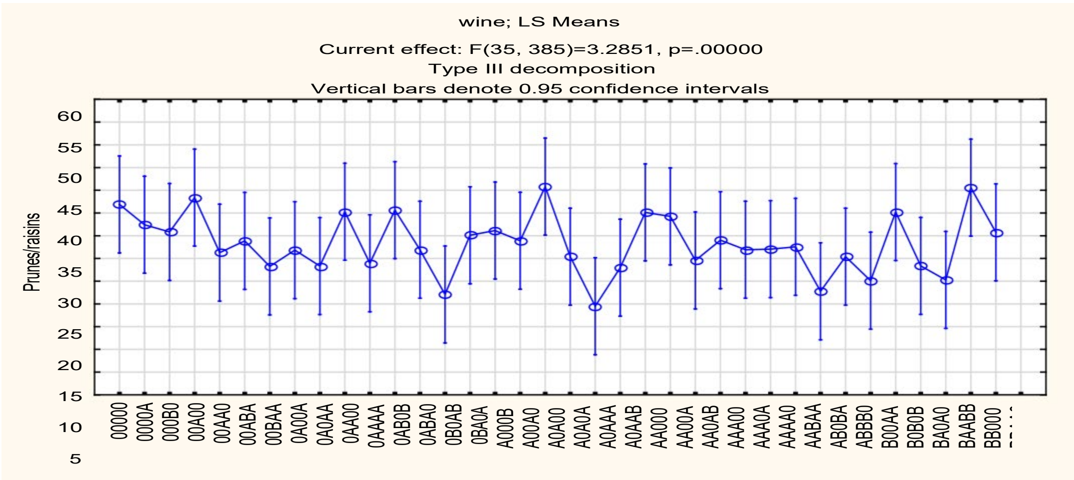


Figure B2 : Least squares means diagrams for attributes prunes/ raisins

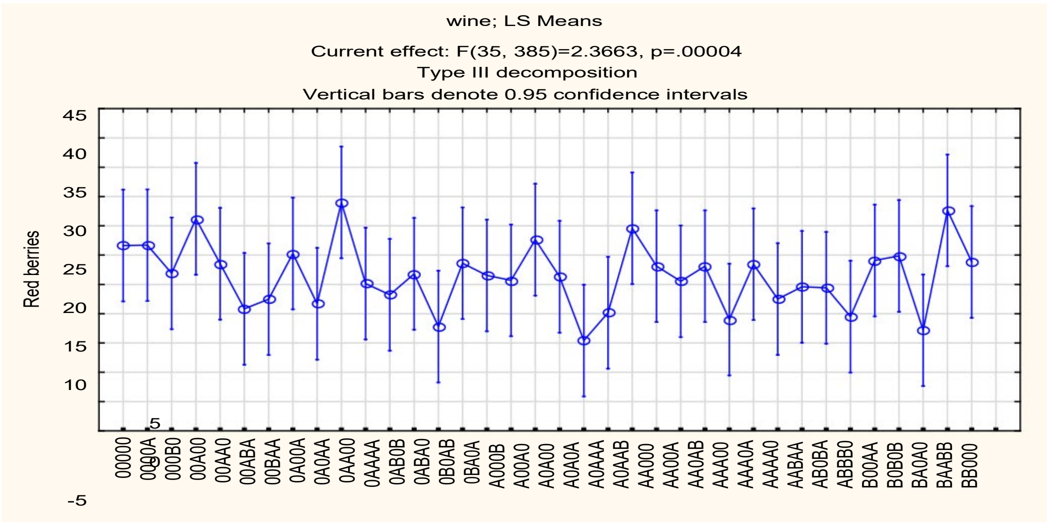


Figure B3 : Least squares means diagrams for attribute red berries

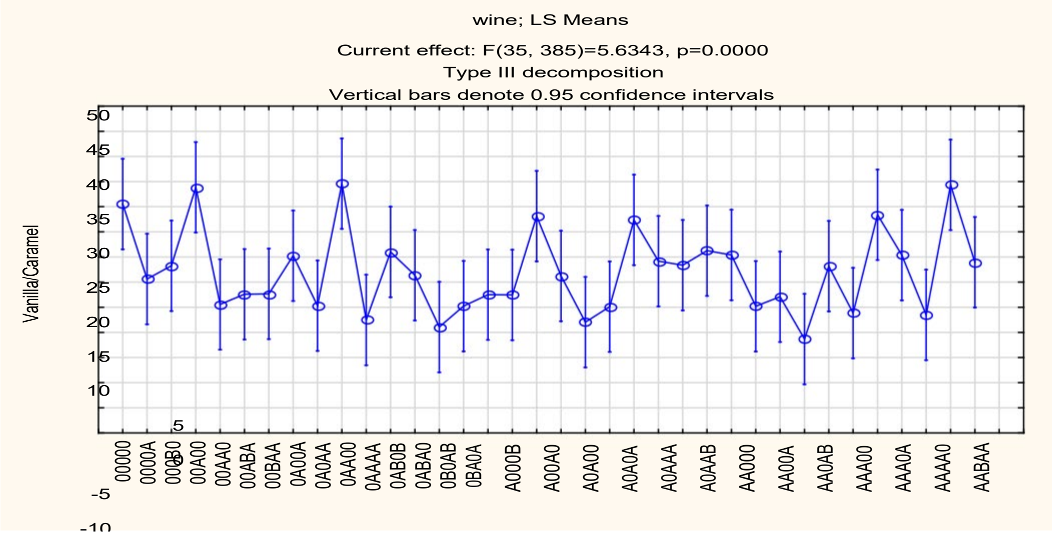


Figure B4 : Least squares means diagrams for attribute ‘vanilla /caramel’

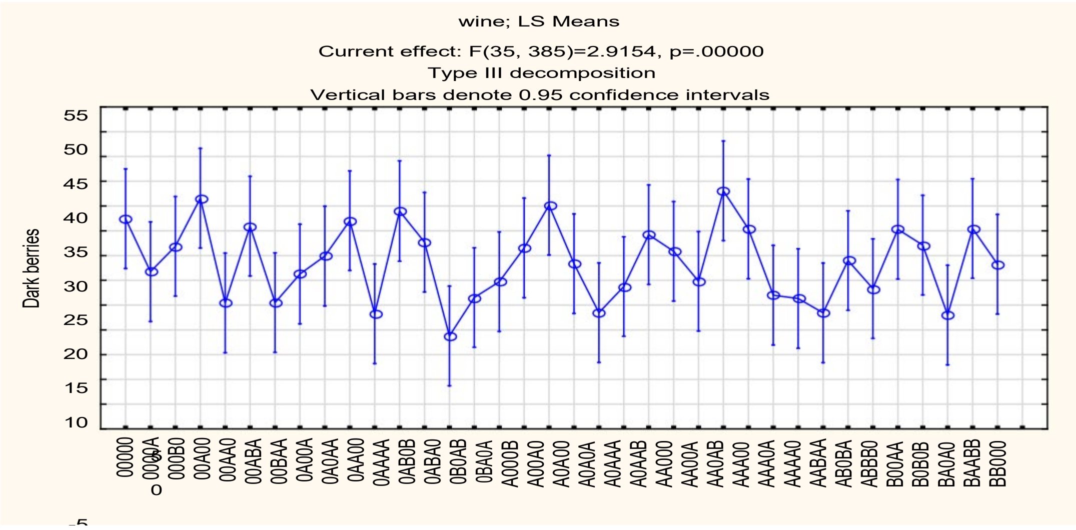


Figure B5 : Least squares means diagrams for attribute ‘dark berries‘

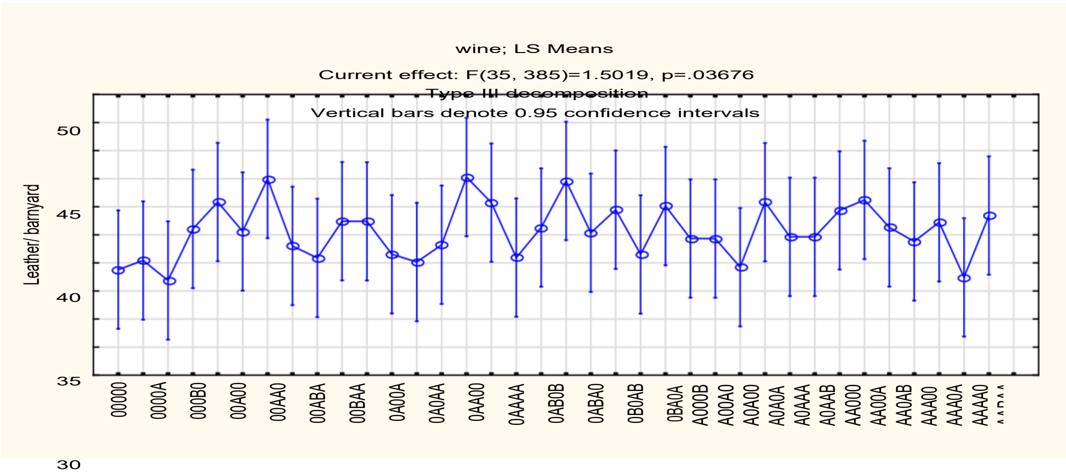


Figure B6: Least Squares Means diagrams for attribute: leather /arnyard

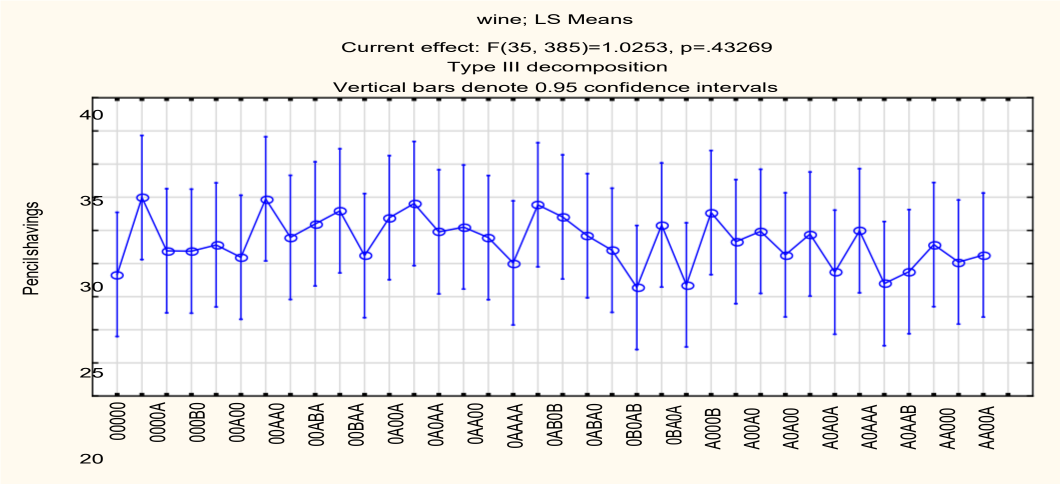


Figure B7 : Least squares means diagrams for attribute 'pencil shavings'

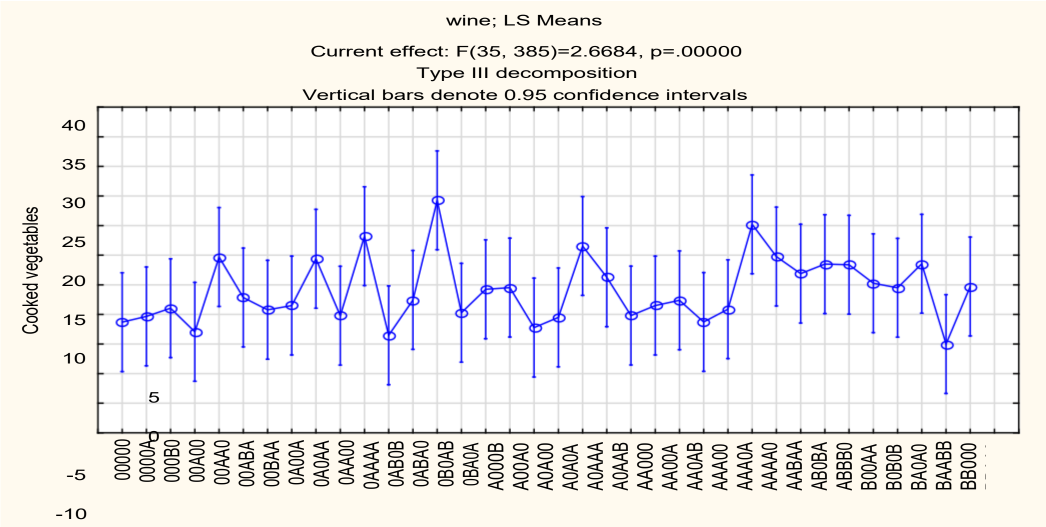


Figure B8 : Least squares means diagrams for attribute 'cooked veg'

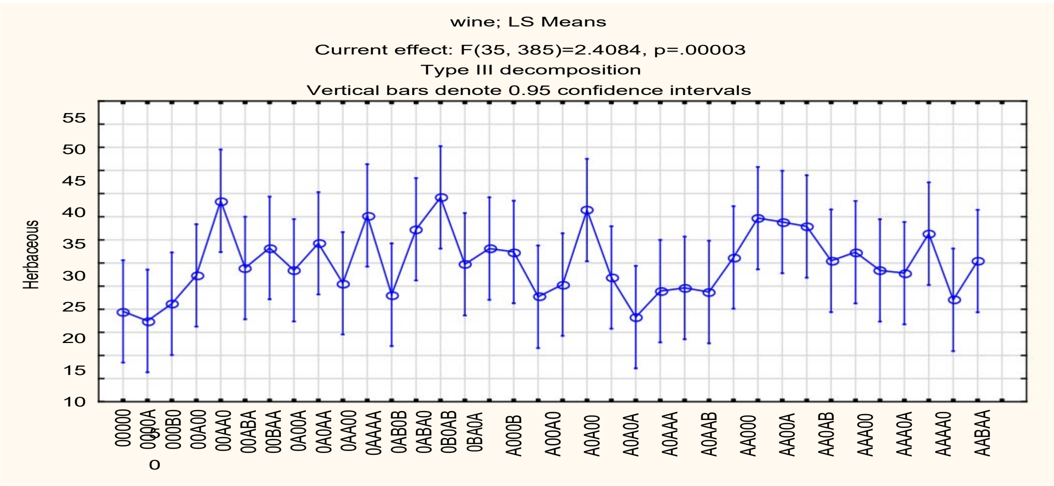


Figure B9 : Least squares means diagrams for attribute 'herbaceous'

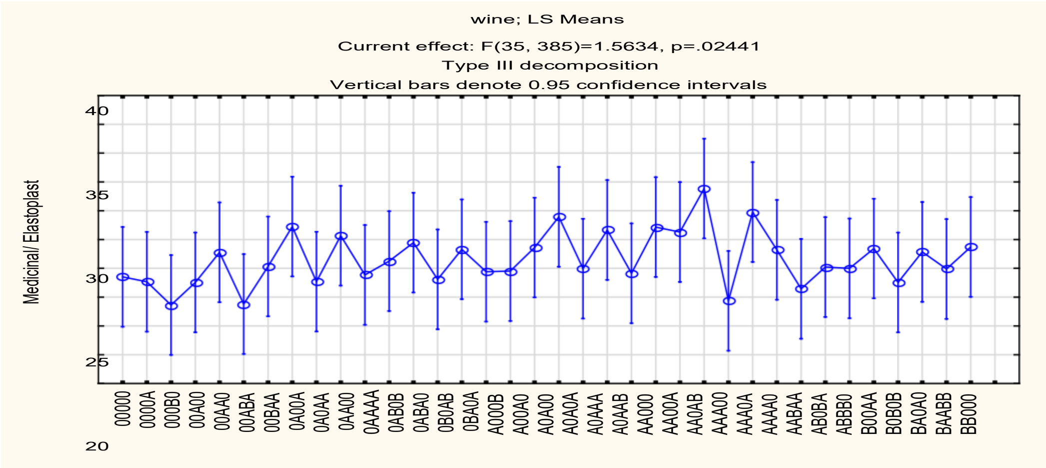


Figure B10 : Least squares means diagrams for attribute ‘medicinal/Elastoplast

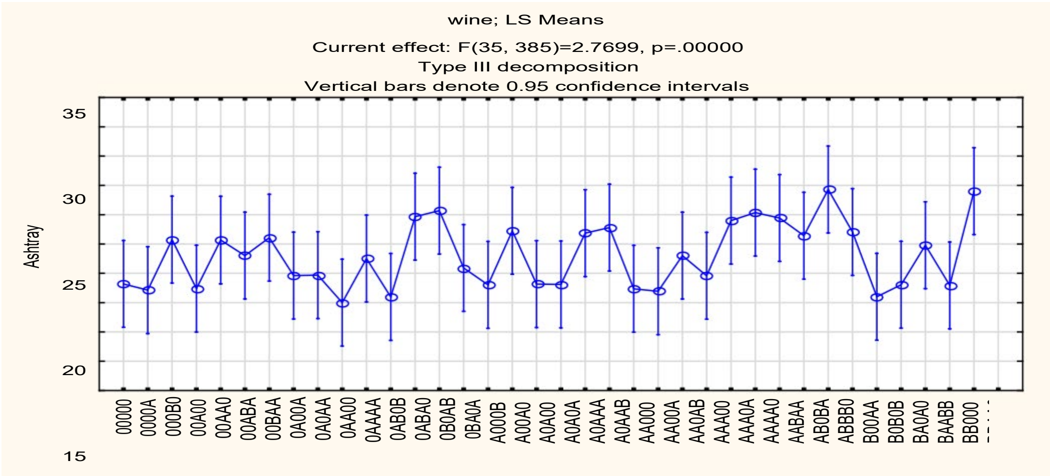


Figure B11: Least squares means diagrams for attribute ‘ashtray’

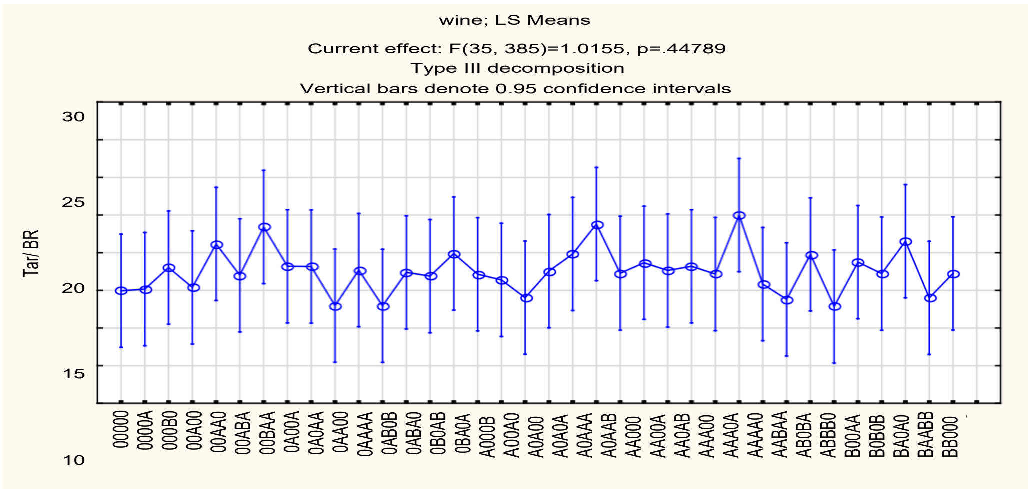


Figure B12 : Least squares means diagrams for attribute ‘Tar/BR

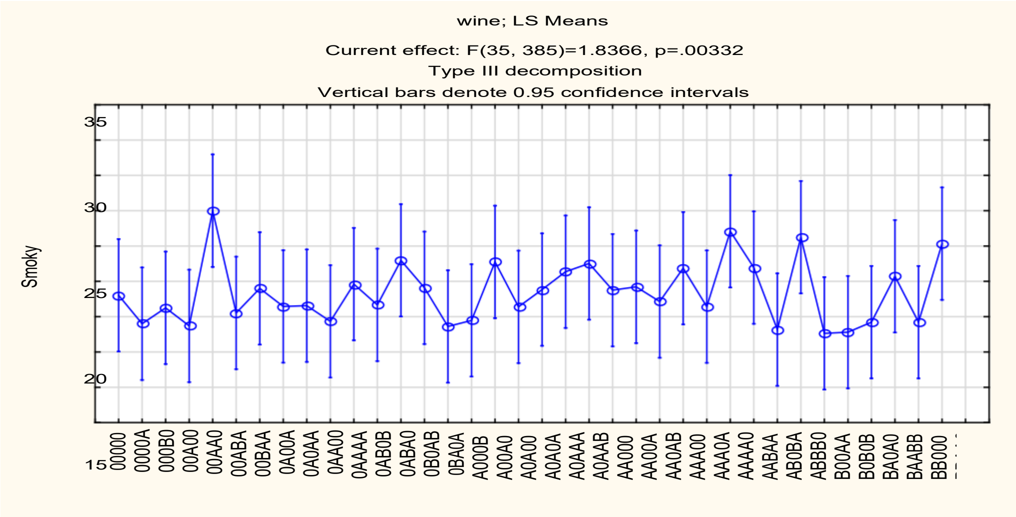


Figure B13: : Least squares means diagrams for attribute 'smoky'

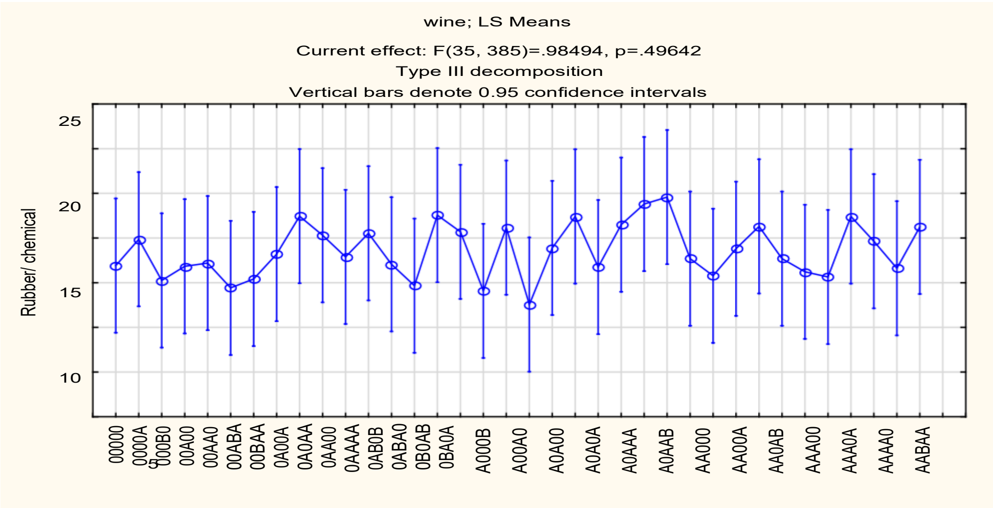
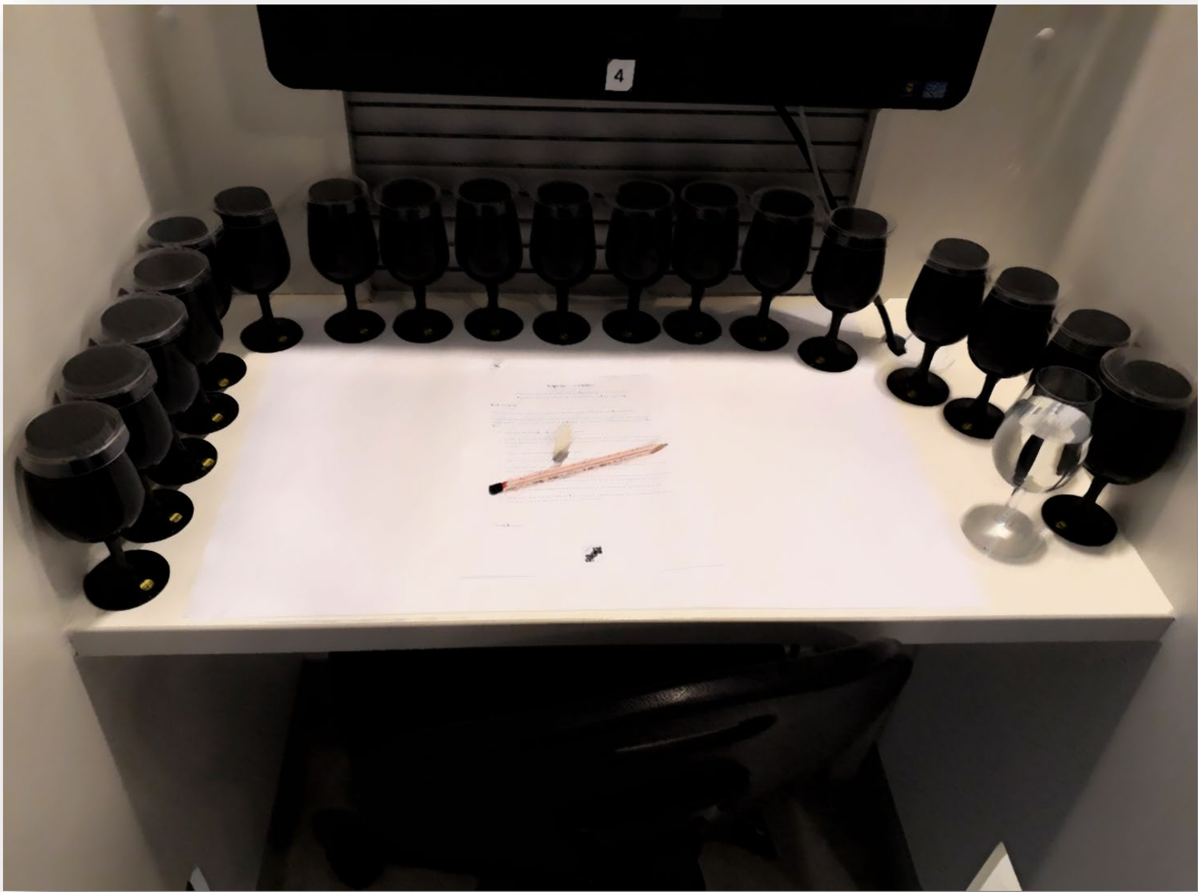


Figure B14: Least squares means diagrams for attribute 'rubber/chemical

Chapter 7



Research Results: Comparison of Descriptive Analysis vs Projective Mapping for characterising interactions between four taint off-flavour associated compounds

Comparison of Descriptive Analysis vs Projective Mapping for characterising interactions between four off-flavour associated compounds

Abstract

This study compared the use of Projective Mapping (PM) and Descriptive Analysis (DA) in assessing olfactory interactions in comparatively large (n=18) sample sets in red wine. Sensory results from the DA study of perceptual interactions for four compounds (guaiacol, *o*-cresol, 4-ethylphenol (4-EP) and 2-isobutyl-3-methoxypyrazine (IBMP)) at two different levels (peri- and subthreshold) were compared to results from a PM exercise for a corresponding sample set. Results showed that similar descriptors were generated by the panel for equivalent samples using PM and DA. Samples containing only volatile phenols (VPs) separated from samples containing combinations of VPs and IBMP, with more positive (sweet, fruity) attributes associated with the single compounds and binary combinations. The IBMP-containing samples with higher numbers of VP components were associated with 'earthy' 'potato skin', 'ash', 'tar' and 'green' descriptors. The first two dimensions/components in the correspondence analysis for the PM (61%) explained variability in the dataset to a similar extent to DA (65%). A rich and comparable set of descriptors was generated by both methods. These results thus confirm that related work on VPs in red wine matrices can be carried out with a trained panel using PM rather than DA in future studies.

1. Introduction

Descriptive analysis is a widely used tool in the field of sensory analysis (Kemp *et al.*, 2018). Despite the flexibility, versatility and rigour of the method, it is time-consuming, and relies entirely on regular, diligent attendance by a large panel. DA can become expensive, particularly in studies where complex samples with small differences are being assessed. Intensive training, under the guidance of a highly skilled panel leader is required in order to achieve consensus between panel members regarding the quality and intensity of perceived attributes. Perrin *et al.,* (2007) noted that in the wine industry, these classical methods of sensory analysis (including DA) are not significantly adapted to the specific context, and that winemakers are often not suitable as sensory panellists for intensive training on extended studies. Pickup *et al.,* (2018) noted that the extensive time and cost associated with conventional sensory profiling methods has spurred sensory researchers to develop alternative rapid methods, such as Flash Profiling, and methods based on sorting like and unlike samples, for example, Projective Mapping (PM), or Napping®, and Polarised Projective Mapping (PPM). These latter methods have in common that they generate two-dimensional sensory maps using untrained panellists to separate samples based on perceived sensory similarities (Meilgaard *et al.*, 2016).

Projective Mapping (PM) consists of positioning products on a sheet of blank paper (a tablecloth or ‘‘*nappe*” in French) simultaneously in such a way that two wines are very near each other if they are perceived as similar and distant from one another if they are perceived as different. Each judge chooses their own criteria and the relative importance given to them, and thus PM allows a researcher to collect Euclidian configurations for each subject rapidly. Evaluations of this method have been restrained to manufactured/formulated food models, and predominantly structured on comparisons against the conventional descriptive method, but Kemp *et al.,* (2018), noted that PM is suited to a wine industry context because of its spontaneous aspect and its flexibility. In fact, Lawrence *et al.,* (2013) espouse a ‘free comments’ method for wine professionals stating that ‘methods classically used for sensory analysis appear to be poorly suited to a professional wine panel, which is less time available than other panel types’.

Even though PM is relatively simple to carry out, data analysis can be time consuming as coordinates of the product placements on the sheet need to be measured, and data has to be treated by a multi-block analysis such as generalised Procrustes analysis or another multiple factor analysis method (Tomic, 2013). The descriptive aspect of PM generates a large number of descriptors that need interpretation and combining by a sensory or product expert. As Meilgaard, et al (2016) noted, this may “colour the results” more with the analyst’s perspective than the panellists’ verdicts. Also PM does not characterise the products and has to be combined with a descriptive method, or little will be known of the qualitative aspects of the product. Pickup *et al.,* (2018) compared Napping®-Ultra Flash Profiling (UFP) ($n = 72$) against Descriptive Analysis (DA; $n = 8$) and physiochemical measurements with apples and showed that sample configurations generated by DA and Napping®-UFP were not significantly correlated ($RV = 0.425$, $P = 0.077$); but both sensory methods (particularly DA) correlated well with a product map based on chemical analysis of the samples ($P < 0.05$). In the study, apple sample characterisations from DA and Napping®-UFP were driven by different sensory attributes, but the authors felt that the findings supported the use of Napping®-UFP. They concluded that the rapid method exhibited strengths in generating information based on holistic perception of products. Wilson (2017) notes that an important limitation of both PM and DA is the number of samples that can be tested. The suggested number for testing is 12 for both methodologies (SSP, 2018) as the cost and time needed for training and testing increase with increasing sample set size. This authors stated that although limitation of sample size is not a problem for studies which investigated differences between controls and a few treatments, if larger sample sets are required, the process can become challenging and expensive.

Rationale and motivation: This experiment was conceived to validate the use of PM in wine by comparing results from a complex interaction study for four compounds (guaiacol, *o*-cresol, 4-ethylphenol (4-EP) and 2- isobutyl-3-methoxypyrazine (IBMP) at two different levels (peri- and subthreshold) that had been previously carried out on a similar sample set using DA, using a de-aromatised Shiraz base wine. The study set out to assess whether a similar attribute set was generated for both methods for the sample set as a whole, and that there was sufficient agreement between PM and DA in terms of the attributes associated with particular samples and compound combinations. The study also included a larger than recommended sample set of n=18 for PM, and n=15 for DA.

2. Materials and Methods

2.1 Panel Selection

Eleven panellists participated in each experiment and the same judges were used for all testing in both studies (DA and PM). All judges were non-smoking females between the ages of 24 and 60. Judges took part in a simplified study to confirm odour detection thresholds for these compounds (McKay *et al.,* 2018) as the published ODTs were determined in different matrices than that of the study. Judges had previous experience in the use of descriptive analysis and sorting, and were shown to be sufficiently sensitive to the compounds under investigation.

2.2 Base wine

An unwooded 2016 Shiraz wine was partially de-aromatised with activated charcoal powder (Merck, Darmstadt, Germany) following the method outlined by Wilson *et al.,* (2018) prior to the wine being used for the sub-threshold olfactory interaction studies. Analysis of volatile phenols in the wine was performed by gas chromatography-mass spectrometry (GC-MS) following the method outlined by De Vries *et al.,* (2016). The guaiacol level in the base wine was 1.37 µg/L, *o*-cresol was 0.08 µg/L and 4-ethylphenol concentration was 1.4 µg/L.

2.3 Preparation of spiked wine samples

Details of the preparation of stock solutions and spiking quantities of the four compounds are specified in Chapter 5 (*Perceptual interactions and characterisation of odour quality of binary mixtures of volatile phenols and IBMP in a red wine matrix*) and Chapter 6: (*Investigation of perceptual olfactory interactions of low levels of five off-flavour causing compounds in a red wine matrix)* of this thesis.

The volatile phenols were added at peri-threshold (A) and subthreshold (B) levels to a partially de-aromatised red wine matrix. The de-aromatised wine was deemed free of volatile phenols by GC-MS analysis (McKay, *et al*., 2018). Therefore, compounds were added at their ODTs ("peri-threshold" level), or at 60-70% of their ODT ("subthreshold" level). Guaiacol, with an odour detection threshold (ODT) in red wine of 23 µg/L (Parker *et al.*, 2013), was added at that level, and at the subthreshold level 15 µg/L. The second volatile phenol, *o*-cresol was added at its ODT of 62 µg/L

(Parker *et al.*, 2013), and at a subthreshold level of 40 µg/L. 4-EP was added at its ODT of 605 µg/L (Chatonnet *et al.*, 1992), and at the subthreshold level of 400 µg/L. IBMP has an ODT of 15 ng/L (Roujou De Boubée *et al.*, 2000) which was considered, during benchtop evaluation, as being too strong for the de-aromatised matrix and was therefore spiked at two subthreshold levels: 10 ng/L and 7ng/L.

Table 1 shows samples codes and spiking regimes (combinations of compounds) for samples in the DA and PM studies, indicating which samples were present (1) or absent (0) in each sensory evaluation.

Table 1. Spiking regime for 18 samples with four compounds for the DA and PM analysis (spiked volumes for all compounds in µg/L, except IBMP in ng/L)

Sample code	DA study	PM study	Guaiac ol	*o-* Cresol	4-EP	IBMP
0000	1	0	0	0	0	0
000A	0	1	0	0	0	10
00A0	1	1	0	0	605	0
000B	1	0	0	0	0	7
0AA0	1	1	0	62	605	0
00AA	1	0	0	0	605	10
0B00	0	1	0	40	0	0
0ABA	1	1	0	62	400	10
0A0B	0	1	0	62	0	7
0BAA	0	1	0	40	605	10
A000	0	1	23	0	0	0
A0A0	1	1	23	0	605	0
AA00	1	1	23	62	0	0
A00A	1	0	23	0	0	10
A0AA	0	1	23	0	605	10

Table 1 (cont.)

AA0A	0	1	23	62	0	10
AAA0	1	1	23	62	605	0
AAAA	1	1	23	62	605	10
ABBB	1	1	23	40	400	7
BA0A	1	0	15	62	0	10
BAB0	0	1	15	62	400	0
B0AB	0	1	15	0	605	7
BB0A	0	1	15	40	0	10
BB00	1	0	15	40	0	0
BBAA	1	0	15	40	605	10

2.3.1 DA samples

Preparation and testing of samples using DA took place in a prior study *(Chapter 6: Investigation of perceptual olfactory interactions of low levels of five off-flavour causing compounds in a red wine matrix*). Results generated during the perceptual interactions study were reanalysed for the purposes of comparison with PM data. The data (n= 15 samples-Table 2) were subject to a post- hoc statistical analysis to generate correspondence analysis plots.

2.3.2 PM samples

For the PM study, eighteen wine samples were spiked with combinations of the five compounds, following a partial D-optimal design constructed with Statistica 12. Sample codes indicate which compounds the sample contained, and at what levels. Position 1 indicates guaiacol, at peri- (A) or sub- (B) threshold levels. Position 2 indicates *o*-cresol, position 3 indicates 4-EP and position 4 indicates IBMP. Thus, to explain the coding: the sample with sample code 'AB0B' was spiked with guaiacol at peri- threshold level (23 µg/L), *o*-cresol at sub-threshold level (40 µg/L), no 4-EP was spiked, and IBMP was at sub-threshold level (7 ng/L). Base wine was spiked within 24 hours of sensory analysis and stored at 5˚C in the dark. Stock solutions were stored at 5˚C in brown, sealed glass bottles, with the exception of the IBMP stock solution which was stored at -20˚C in foil- wrapped containers to prevent light incursion.

2.4 Sensory Testing

A single sensory modality in all samples (odour) was evaluated in both experiments, DA and PM. All sensory testing took place in individual sensory booths in a well-ventilated, odorless facility (ISO 8589: 2007) with standard artificial daylight lighting and temperature control at 20 ± 1°C. Products were presented in a different randomised order for each panellist according to a Williams Latin Square design (Macfie *et al.*, 1989). Coded wines were presented to judges as in black (ISO 3591:1977) standard glasses and covered with plastic lids. All glasses were prepared one hour before serving to allow for temperature and headspace equilibration. Communication was not allowed between the judges for the duration of the test.

2.4.1 DA testing

DA testing was carried out as outlined in Chapter *4 (Investigation of perceptual olfactory interactions of low levels of five off-flavour causing compounds in a red wine matrix).* For the original DA study, 36 samples were evaluated, and a subset of sensory results for 15 samples which contained the four compounds of interest were reanalysed for the purposes of comparison with results from PM.

2.4.2 PM testing

Risvik *et al.*, (1997) indicated that three replicates of a projective mapping exercise usually produced similar maps, at least on the first two dimensions. Even though the suggested number of samples to be presented to panellists is 12 (Pagès, 2005) prior discussion with the panel established that they were willing to sort 18 wines on one occasion, as it was only a single event (no replicates on the same day), and there was no tasting (gustation) involved. The PM exercise was carried out on three consecutive days in a sensory laboratory approximately four months after the DA exercise. Panellists were completely unaware that they were testing the same sample set as had been tested with DA. They were provided with verbal and written instructions (Appendix 1A), and were asked to evaluate all 18 wines, based on similarities and dissimilarities of aroma. Samples were positioned on an A2 (42 cm x 60 cm) sheet of paper in accordance with their similarity or differences. They were allowed to reassess wines as many times as needed according to their own criteria. When they had finished evaluating the wines, and had decided on the final positions of the samples in relation to each other, they were asked to mark the positions of each sample code on the paper. Additionally, panellists were asked to write three descriptors for each wine on a sticky note, and place this next to the code for the wine. Due to the number of the wines, and in line with the recommendation of (Pickup *et al.*, 2018) the descriptive step was performed at the same time as the arranging step to assist with panellists' memory of each wine's main aroma attributes.

2.5 Data analysis

For the DA data, the averages of the triplicate measurement of each attribute for each judge was used for statistical analysis. A Principal Component Analysis (PCA) was obtained using the correlation matrix of the mean data with R (R Core Team, 2015). Data were centred and scaled for each attribute using estimated mean and variance respectively. For the PM study sample locations on the A3 sheet were measured manually as (X,Y) coordinates from the bottom-left corner of each judge's sensory 'map'. Coordinates (X,Y) were measured for each sample, and results entered into an Excel (Windows, Microsoft Corporation) table. As there was duplication and overlap in the descriptors allocated to each wine, the list of around 50 attributes generated was condensed systematically to main groupings. If a descriptor was cited by fewer than 20% of the judges, it had to be combined with a similar term (Campo *et al.*, 2005). The groupings included 'berry fruits', 'fruity (other)', 'sweet-associated', 'floral', 'jammy' 'prunes/raisins', 'plums' 'spicy', 'savoury', 'smoky', 'ashy', 'chemical', 'Rubber/BR, 'tar', 'green/fresh' 'cooked/vegetal', 'animal', 'Oak/wood', 'toasted', 'earthy', 'dusty', tobacco/cigar box', 'medicinal', 'barnyard', 'leather', 'acetone', 'alcohol' and 'plastic'. Correspondence analysis (CA) and multiple factor analysis (MFA) were carried out using R (R Core Team, 2015), with panellist's product coordinates as variables. For all of the data, the effects were considered significant when $p < 0.05$.

3. Results and discussion

3.1 Descriptive analysis:

The Principal Component Analysis (PCA) biplot of the DA study of attributes associated with samples (n=15) spiked with combinations of three VPs and IBMP is shown in Figure 1. The first principal component explains 53% of variation, and the second 12%. Thus, 65% of the variation in the dataset is explained by the two principal components, which is satisfactory for a sensory study.

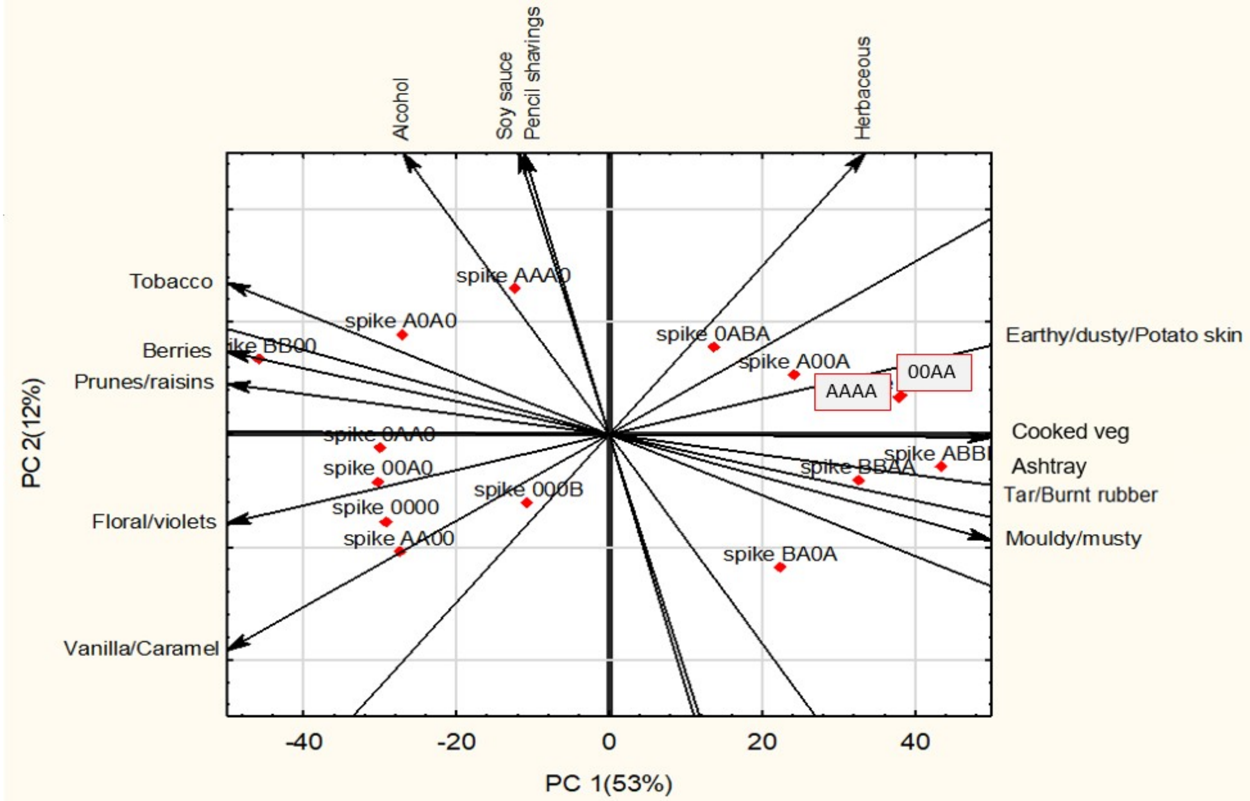


Figure 1. PCA biplot of the general sensory results of attributes of samples (n=15) spiked with combinations of the three VPs, and IBMP in dearomatised Shiraz wine in the following order: (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) at 0, peri (A) and subthreshold (B) levels.

Samples spiked with only the VPs (guaiacol (position 1), *o*-cresol (position 2), 4-EP (position 3)), as single and binary components are grouped towards the negative PC1 of the biplot. These samples include the control (0000), as well as BB00, A0A0, 00A0,and 0AA0.These samples are associated with attributes such as ‘vanilla/caramel’, ‘floral violets’, ‘prunes/raisins’ and ‘berries’ on the negative side of PC1 on the biplot. Samples combining VPs with IBMP (position 4) at peri- threshold levels (including 00AA, AAAA, ABBB, BBAA) are aligned on the positive side of PC1. These samples are associated with ‘earthy/dusty/potato skin’, ‘cooked veg’, ‘ashtray’, ‘mouldy musty’ and ‘tar/BR’ attributes indicating that perceptual interactions of these combinations lead to generally negative descriptors. These samples generally contained a higher number of compounds. The clear separation along PC1 between samples suggests agreement between panel members on odour attributes.

3.2 Projective mapping

The CA plot of the PM data for the sensory study of attributes associated with samples (n=18) spiked with combinations of volatiles phenols and IBMP is shown in Figure 2. Over 60% (Dimension 1 = 50.9%, Dimension 2 = 9.5%) of the variation between the samples is explained by first two dimensions. Samples located along the positive axis of Dim 1 (BAB0, AAA0, AA00, 0B00, A000 and

00A0) are characterised as 'jammy', 'sweet-associated', 'oak/woody', 'prunes/raisins', 'berries', and 'floral/perfume'.

Samples located along the negative axis of Dim 1 (including ABBB, A0AA, AA0A, 0BAA, AAAA, and BB0A) are associated with 'earthy' 'potato skin', 'ash', 'tar' and 'green' (cooked and fresh) descriptors. Samples containing complex combinations of IBMP in combination with volatile phenols are opposed along Dimension 1 to samples containing only volatile phenols and simpler configurations. This is similar to the results obtained with DA, and even though the explained variance is approximately 60% compared to the 65% of DA, the satisfactory separation and percentage of explained variance along the first dimension indicate consensus among the panellists on differences and similarities between samples.

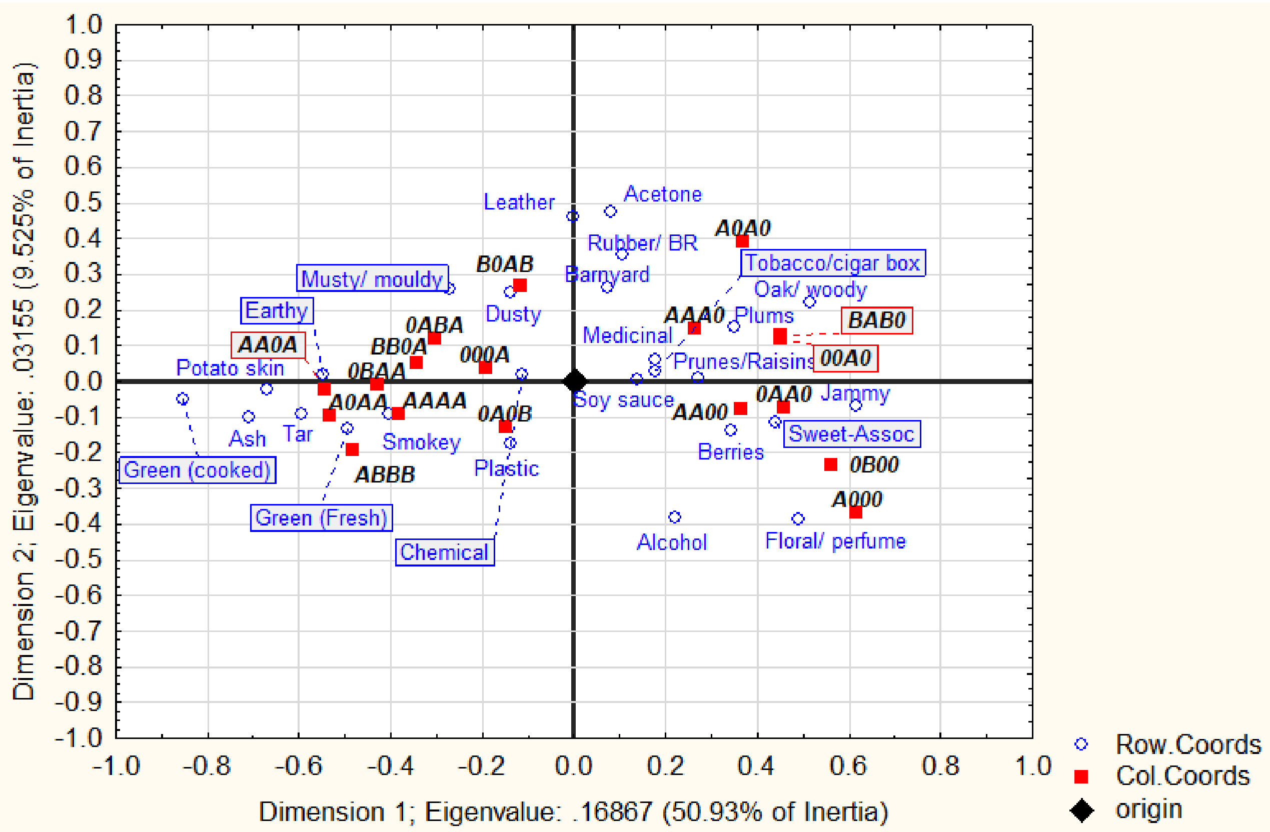


Figure 2. Correspondence Analysis 2D plot for standardised row and column profiles for the PM study of general sensory results of samples (n=18) spiked with combinations of the three VPs, and IBMP in dearomatised Shiraz wine in the following order: (position 1: guaiacol), (position 2: o-cresol), (position 3: 4-EP), (position 4: IBMP) at 0, peri (A) and subthreshold (B) levels

4. Conclusions

This study compared the use of a rapid Projective Mapping (PM) method and classical Descriptive Analysis (DA) in assessing olfactory interactions in red wine using the same sensory panel. Results showed that similar attributes were generated by the panel for both DA and PM sample sets, with good consensus regarding sample attributes amongst panel members for both methods. In both sensory evaluation methods, samples containing only volatile phenols (VPs) separated out from samples containing combinations of VPs and IBMP along Dimension 1. Samples with only VPs were associated with more positive (sweet, fruity) attributes associated with the simpler combinations, and the more complex, IBMP-containing samples associated with 'earthy' 'potato skin', 'ash', 'tar' and 'green' descriptors. The first two dimensions/components in the PM analysis explained 61% of the variance, which was not as good as 65% explained by the first two components in the DA, but a comparable set of descriptors was generated, in much less time. These results thus confirm that related work on VPs in red wine matrices in future studies can be carried out with a trained panel using PM rather than DA.

REFERENCES

Campo, E., Ferreira, V., *et al*., 2005. Prediction of the Wine Sensory Properties Related to Grape Variety from Dynamic-Headspace Gas Chromatography−Olfactometry Data. J. Agric. Food Chem. 53, 14, 5682–5690.

Chatonnet, P., Dubourdie, D., *et al*., 1992. The origin of Ethylphenols in wines. J. Sci. Food Agric. 60, 2, 165–178.

ISO, 1977. ISO 3591:1977 - Sensory analysis -- Apparatus -- Wine-tasting glass. Available at https://www.iso.org/standard/9002.html.

ISO, 2007. ISO 8589:2007 - Sensory analysis -- General guidance for the design of test rooms. Available at https://www.iso.org/standard/36385.html.

Kemp, S.E., Ng, M., *et al.,* 2018. Introduction to Descriptive Analysis: Descriptive. Analaysis and. Sensory Evaluation. John Wiley & Sons, Ltd, Chichester, UK 1–39.

Lawrence, G., Symoneaux, R., *et al*., 2013. Using the free comments method for sensory characterisation of Cabernet Franc wines: Comparison with classical profiling in a professional context. Food Qual. Prefer. 30, 2, 145–155.

Macfie, H.J., Brathchell, N., *et al*., 1989. Designs to balance the effect of order of presentation and first- order carry-over effects in Hall Tests. J. Sens. Stud. 4, 2, 129–148.

McKay, M., Bauer, F., *et al*., 2018. Testing the Sensitivity of Potential Panelists for Wine Taint Compounds Using a Simplified Sensory Strategy. Foods 7, 11, 176.

Meilgaard, M.C., Civille, G., *et al*., 2016. Sensory evaluation techniques. (Fifth ed.). CRC Press, Taylor & Francis Group, New York.

Pagès, J., 2005. Collection and analysis of perceived product inter-distances using multiple factor analysis: Application to the study of 10 white wines from the Loire Valley. Food Qual. Prefer. 16, 7, 642–649.

Parker, B., Baldock, G., *et al*., 2013. Seeing through smoke. Wine Vitic. J. January/Fe, 28, 42–46.

Perrin, L., Symoneaux, R., *et al*., 2007. Comparison of three sensory methods for use with the Napping procedure: Case of ten wines from Loire valley. Food Qual. Prefer. 19, 1–11.

Pickup, W., Bremer, P., *et al*., 2018. Comparing conventional Descriptive Analysis and Napping®-UFP against physiochemical measurements: a case study using apples. J. Sci. Food Agric. 98, 4, 1476– 1484.

Risvik, E., McEwan, J., *et al*., 1997. Evaluation of sensory profiling and projective mapping data. Food Qual. Prefer. 8, 1, 63–71.

Roujou De Boubée, D., Van Leeuwen, C., *et al.,* 2000. Organoleptic Impact of 2-Methoxy3-isobutylpyrazine on Red Bordeaux and Loire Wines. Effect of Environmental Conditions on Concentrations in Grapes during Ripening. J. Agric. Food Chem. 48, 4830–4834.

SSP, 2018. Projective Mapping Available at https://www.sensorysociety.org/knowledge/sspwiki/Pages/Projective Mapping.aspx.

Tomic, O., 2013. Differences between generalised procrustes analysis and multiple factor analysis in case of projective mapping. Master's Thesis. Norwegian University of Life Science, Norway.

De Vries, C., Mokwena, L., *et al.*, 2016. Determination of Volatile Phenol in Cabernet Sauvignon Wines, Made from Smoke-affected Grapes, by using HS-SPME GC-MS. South African J. Enol. Vitic. 37, 1, 15–21.

Wilson, C., 2017. Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines, Master's Thesis, : Stellenbosch University, Western Cape, South Africa.

Wilson, C., Brand, J., et al., 2018. Interaction Effects of 3-Mercaptohexan-1-ol (3MH), Linalool and Ethyl Hexanoate on the Aromatic Profile of South African Dry Chenin blanc Wine by Descriptive Analysis (DA). South African J. Enol. Vitic. 39, 2, 271–283.

Appendix 1A:

Napping/Projective Mapping – red wines

Welcome and thank you for participating in this project. Please read the following instructions before starting.

Instructions

Please evaluate the 18 wines according to **AROMA ONLY similarities** and ***dissimilarities.***

Please mark the positions of the wines on the sheet in front of you in such a way that wines with **similar aroma** characteristics are **close to each other** and wines with **different** characteristics **far apart**

- ***Evaluate the AROMA – do not drink the wine!***
- **Smell** the wine and write down the 3-digit number of the wine on a post-it. Write down your immediate impressions; whatever comes to your mind that you think is suitable to describe the wine.
- Please write **3-5 descriptors/ideas per wine**, choosing from the given list of attributes.
- Place the wine glass and post-it on the A2 paper sheet.
- Evaluate the next wine, write the number and descriptors on the post-it and place it on the sheet according to how similar or dissimilar it is from the other wine.
- You can move the wine glasses/post-its around as you evaluate the wines and relate them to one another. You can evaluate and re-evaluate the wines as many times as you wish.
- Please use the **whole sheet** to express your opinion on differences amongst the wines.
- When you have finished make an **X** on the paper and write down the numbers of the wines and descriptors in the place it occupies on the sheet.

Thank you!

Chapter 8

Research Results: Investigating the effects of two volatile phenols on aromatic perception of four red wine cultivars using Projective Mapping

Investigating the effects of two volatile phenols on aromatic perception of four red wine cultivars using Projective Mapping

Abstract:

The qualitative perceptual interactions and effects of two compounds that have been shown to contribute to smoke taint, 4ethyl phenol (4-EP) and *o*-cresol, were tested in combination in four different cultivars (viz. Cabernet Sauvignon, Merlot Noir, Pinotage and Shiraz), by a trained panel of twelve judges using Projective Mapping. Data presented here show that, individually and in combination, *o*-cresol and 4-EP can have a marked effect on the perception of attributes in different cultivars. Although there were marked similarities between the cultivars for certain aspects, there were also subtle but important differences in sensory profiles of the spiked samples. The binary spikes led to definite increases in the perceptions of 'animal', 'tar', 'burnt rubber', 'dusty' 'earthy' across all four cultivars, and very clear separation in the sensory space of the binary-spiked samples from clean controls which were perceived as 'fruity berries', 'fruity-other', 'sweet-associated' and 'floral'. There were differences between the cultivars in response to single volatile phenol spikes. Differences in base wine also showed effects, particularly with Pinotage. This study also emphasises the need for researchers to carefully consider the composition of the matrix when determining and comparing odour thresholds and olfactory perceptual interactions in wine, and the effects of treatments on wine aroma.

1. Introduction

A large number of factors influence the olfactory perception of wine including (but not limited to) the nature and concentration of compounds, the genetic make-up and experience of those judging/perceiving them and the matrix in which the compounds are embedded. Odour detection thresholds (ODTs) reported for aroma compounds frequently do not mention the matrix in which the value was originally estimated (Perry & Hayes, 2016). As the composition of the solution has a profound effect of the release and perception of odour, a volatile compound may thus be obvious in one solution and subtle or undetectable in another. Odour may also change with a change in concentration, and with the presence of other compounds in the matrix, leading to the generation of quite different descriptors for different wines containing the same compound. Perry & Hayes (2016) state that the choice of matrix is a critical consideration when reporting thresholds to prevent 'widely varying and non-generalizable values' from being perpetuated throughout the literature.

In the past, a tendency in the wine industry, particularly in cool climates regions, was to try and achieve higher sugar levels, therefore producing wines with relatively high levels of alcohol (Goldner, *et al.*, 2009). There has been a swing towards lower alcohol wines in recent times driven by consumer requirements (Ozturk & Anli, 2009.; Goode, 2018). This has implications for olfactory perception, as studies have shown that ethanol concentration can influence the relative contribution of aroma compounds to instigate changes. In a study by Goldner *et al*., (2009), wine odour at higher alcohol concentrations was described as 'herbaceous', compared to 'fruity' perceived at lower ethanol levels. Petrozziello *et al.*, (2014) revealed that higher ethanol levels in model wine decreased the volatility of 4-EP, and higher alcohol products were described as more 'fruity' in their study. The 'phenolic' note was also significantly higher in the samples with lower non-volatile polyphenol content. The influence, therefore, of ethanol and polyphenols on the perception of the 'Brett' character (including descriptors 'stable', 'manure', 'horse sweat' and 'phenolic') was increased (Petrozziello *et al.*, 2014).

Interactions between matrices and components are thus complex. Theories of additive and antagonistic effects between odour compounds have been postulated (de March *et al.*, 2015) which state that detection thresholds of compounds can be affected when they are present in solution with other compounds. Perceptual masking (antagonistic) effects may be an important part of understanding the underlying cause of matrix dependence of detection thresholds, and these have also been studied in relation to aroma and taste perception (Rospars *et al.*, 2008; Chaput *et al.*, 2012), but the extent to which these effects influence detection thresholds remains largely unanswered. Perry & Hayes (2016) investigated differences in thresholds evaluated in diverse matrices, using two structurally similar odour-active compounds commonly found in *Vitis Labrusca* wines: methyl anthranilate (MA) and 2-aminoacetophenone (2AAP). They found a detection threshold for 2AAP of 0.5 µg/L in Pinot Gris and Chardonnay and 2.0 µg/L in Riesling (more complex aroma profile of 'floral' and 'petrol') which indicated perceptual masking effects may be an important part of understanding the underlying cause of matrix dependence of detection thresholds. These authors stated that work has also shown there may be a hundredfold difference in detection thresholds for certain structurally similar compounds (Perry & Hayes, 2016). Although it has been found that this theory does not hold true in all cases (Grosch, 2001), additive / synergistic perceptual effects have been shown for specific odour pairs (Laing *et al.*, 1994).

Previous findings of this project (Chapter 3 and Chapter 4) have shown that combinations of volatile phenols (VPs) at peri- and subthreshold levels can lead to the perception of attributes that are not predictable from the descriptors associated with the pure compounds in the literature. The perceptual effects of interactions found in solutions containing combinations of VPs, as well as IBMP and TCA, in de-aromatised red wine (Shiraz) included increases in 'green', 'herbaceous', 'ashy', 'earthy/dusty', 'cooked veg' and 'tar/BR' attributes. The combinations of VPs, IBMP and TCA also decreased perception of sweet and fruity attributes in de-aromatised Shiraz. It was also found (Chapter 5), in accordance with other studies (Perrin *et al*., 2007; Dehlholm *et al*., 2012; Hopfer & Heymann, 2014), that the information gained from projective mapping (PM) was accurate and reliable in assessing the subtle and complex differences induced by these perceptual olfactory interactions.

The rationale for this section of the study was therefore to test mixtures of two VPs (*o*-cresol and 4-EP) at peri-threshold levels in four red wine matrices (Cabernet Sauvignon, Merlot Noir, Shiraz and Pinotage) in order to establish whether the olfactory perceptual effects were consistent or variable across cultivars.

2. Materials and Methods

2.1 Wines for the matrix study

Four common South African red wine cultivars were selected for the study: Pinotage, Shiraz, Cabernet Sauvignon, and Merlot Noir. Two commercial South African examples of each cultivar were sourced with the criteria being that the wines should not be wooded. The wines were less than two years old at the time of sensory evaluation in order to avoid age- associated attributes that might mask or enhance subtle effects caused by the peri- and subthreshold levels of VPs. The wines, purchased in 5 L ‘bag-in-box’ format, are listed in Table 1.

Informal benchtop screening by five experienced sensory judges confirmed that the wines were completely free of any off-odours. The wines all had aroma profiles that were dominated strongly by fruit, which warranted partial de-aromatization with activated charcoal powder (Merck, Darmstadt, Germany) in order to avoid potential masking of subtle interaction effects. De-aromatization was carried out (following the method outlined in Chapter 3) prior to the wine being used in investigations into sub-threshold olfactory effects. In a screening session, the expert panel chose a blend of 50:50 charcoal-treated wine to untreated wine ratio which yielded a neutral wine base with low aromatic intensity. In between testing, the wines were stored in their original packaging at 12 C at the Department of Viticulture and Oenology, Stellenbosch University, South Africa. Analysis of volatile phenols in the wine was performed by gas chromatography-mass spectrometry (GC-MS) following the method outlined by (De Vries *et al.*, 2016). Concentrations of the two VPs in the wines were measured before and after partial de-aromatization, with the latter wines being used for the study.

Table 1. Eight South African red wines of four different cultivars selected for the matrix study

Wine code	Wine of Origin region	Vintage
Shiraz A	Western Cape	2017
Shiraz B	Robertson	2017
Pinotage A	Western Cape	2016
Pinotage B	Robertson	2017
Merlot Noir A	Roberston	2017
Merlot Noir B	Western Cape	2017
Cabernet Sauvignon A	Robertson	2017
Cabernet Sauvignon B	Du Toitskloof	2016

Table 2. Average levels of two volatile phenols (µg/L) as determined by GC-MS instrumental duplicates before and after de-aromatisation

	CS A	CS A	CS B	CS B	SH A	SH A	SH B	SH B	PT A	PT A	PT B	PT B	M A	M A	M B	M B
		PD		PD		PD		PD		PD		PD		PD		PD
o-cresol	20	10	68	6	25	5	16	14	10	7	59	13	16	5	38	5
4 EP	3	1	10	5	7	3	1	1	5	2	2	1	1	0	194	55

(CS = Cabernet Sauvignon, SH = Shiraz, PT= Pinotage and M = Merlot Noir, PD = post de-aromatisation). Shaded columns indicate baseline contamination levels used in the study.

2.2. Preparation of spiked wine samples

Stock solutions of 1000 mg/L of both compounds were prepared in 99.5% ethanol (Merck Darmstadt, Germany). The compounds, 4-EP (99.5% purity), *o*-cresol (99%), were obtained from Merck, (Darmstadt, Germany). The compounds were dissolved in ethanol (10 mL) and then made up to volume with ultra-pure distilled water (Millipore, Bedford, MA, USA) to the concentrations required for spiking, i.e.: 100 mg/L for *o*-cresol and 1000 mg/L for 4-EP stock solution.

The base levels for the compounds of interest were tested chemically to determine the spiking levels as shown in Section 2.1, and the spiking levels were adjusted accordingly. Taking into account the existing level of 4-EP and *o*-cresol in the base wine after de- aromatisation (Table 2, shaded columns), sufficient volume of wine for each training or testing session was spiked with an appropriate volume of stock solution to achieve the concentrations (Appendix 1: Table 1) of each volatile compound required for the matrix study. Base wine was spiked within 24 hours of sensory analysis and stored at 5˚C in the dark. Stock solutions were stored at 5˚C in brown, sealed glass bottles. Samples were prepared the day before each training and testing session.

2.3 Panel Selection

The panel consisted of twelve judges, all non-smoking females between the ages of 24 and

60. Judges had previous experience in the use of quantitative descriptive analysis, and experience in smoke taint evaluation in wine. Most of the panellists also took part in the determination of odour detection thresholds for these compounds and therefore already had familiarity with the two compounds under investigation.

2.4 Sensory Training

Even though PM does not normally necessitate any training, a combination of consensus and ballot training was conducted before testing in two training sessions of two hours in order to avoid the generation of excessive numbers of descriptors, and associated issues with data analysis (Mafata *et al.*, 2018). Eight samples from the design (Appendix A, Table 2) as well as two clean controls were discussed in the training sessions. These discussions generated a comprehensive list of descriptors that included the familiar attributes, but also new attributes that were unique to the wines under study (Appendix A, Table 1). The thirteen attributes groups which covered the majority of descriptors, agreed upon by the panel, included: ‘berry fruits’, ‘fruity (other)’, ‘sweet-associated’, ‘floral’, ‘spicy’, ‘savoury’, ‘smokey/burnt’, ‘chemical/plastic’, ‘tar/burnt rubber’, ‘green/vegetal’, ‘animal’, ‘woody/toasted’, and ‘earthy/dusty’.

2.5 Sensory Testing

This study was carried out using PM for two wines each of four different cultivars (Pinotage, Shiraz, Cabernet and Merlot Noir) representing eight different wine ‘matrices’ (Table 1). Testing per cultivar was carried out on different days, with the three technical repeats on the same day. Testing was conducted in individual booths in a sensory laboratory equipped with standard artificial daylight lighting and temperature control at 20 ± 1°C. Coded wines were presented to judges in black (ISO 3591:1977) glasses and covered with plastic lids. Samples were presented using unique three digits blinding codes and randomised according to a Williams Latin Square design (Macfie *et al.*, 1989). All samples were poured 30 minutes before testing to allow for temperature and headspace equilibration. Communication was not allowed between the judges for the duration of the test.

Judges were asked to evaluate the eight wine samples according to similarities and dissimilarities of aroma attributes, and mark the positions of the wines on an A2 (42 x 60 cm) sheet of white paper. They were also asked to write 3-5 descriptors/attributes per wine on a 'post-it' note, choosing from a given list of grouped attributes (Appendix A, Table 2) as outlined in the study by Mafata *et al.,* (2018). The tasting sheets collected at each test session contained the marked locations of each sample and the respective attributes chosen for each of them.

2.6 Data analysis

Data points were assigned two-dimensional coordinates (X, Y), with the origin placed at the bottom left corner of the paper sheet. Results, in the form of a combination of coordinates and attributes for each sample, were entered into an Excel (Windows, Microsoft Corporation) table. Each of the individual data sets were normalised and the data combined to produce a single Correspondence Analysis map per cultivar with Statistica 13.4 (TIBCO Software Inc., Palo Alto, USA). Cochran's Q-tests were used to evaluate significant differences between attributes within cultivar sets.

3. Results and Discussion

Sensory results of the PM for the eight different wine matrices were submitted to Cochran's Q-test (Appendix Table 2-5) in order to test the hypothesis that the occurrence of a descriptor was equal across all four cultivars. If $p<0.05$, the descriptor occurred more with some samples than others. A summary of results for all the cultivars (Table 3 below) show that certain attributes were not significantly different across all the samples in a cultivar; for example, for Cabernet Sauvignon, 'berry', 'spicy', 'ashy', 'chemical', 'plastic' and 'vegetal' attributes were not significantly different across all samples. The 'savoury', and 'earthy attributes were significantly different only at the $p < 0.1$ level. 'Sweet-associated', 'animal', 'woody' and 'tar' attributes were significantly different at the $p<0.001$ level across samples of Cabernet Sauvignon, indicating that these attributes were affected by the treatments. There was no general consistency or patterns in the perception of attributes across the cultivars although Cabernet and Shiraz did seem to show some similarities in the patterns of attributes affected. The perception of attributes will be discussed per cultivar in order to clarify effects of the single and binary spiking effects of volatile phenols.

Table 3. Cochran's Q-test results showing significance of attributes across four cultivars (red colour indicates $p<0.05$; blue indicates $p<0.10$)

	Cabernet Sauvignon		Shiraz		Pinotage		Merlot Noir	
	Q stats	p-val	Q stats	p-val	Q stats	p-val	Q stats	p val
berry	6.89	0.44	21.52	<0.001	8.69	0.28	17.72	0.01
fruity	19.05	0.01	18.20	0.01	12.41	0.09	5.47	0.60
Sweet- assoc.	38.89	<0.001	18.40	0.01	21.06	<0.001	22.38	<0.001
floral	16.02	0.02	20.30	<0.001	5.27	0.63	13.66	0.06
spicy	6.05	0.53	17.10	0.02	5.28	0.63	7.18	0.41
savoury	13.00	0.07	5.98	0.54	16.77	0.02	8.42	0.30
smoky/burnt	17.38	0.02	23.00	<0.001	25.04	<0.001	17.93	0.01
ashy	7.73	0.27	10.23	0.18	8.71	0.27	9.85	0.20
chemical	11.41	0.12	10.46	0.16	18.45	0.01	4.58	0.71
plastic	2.92	0.89	7.64	0.37	9.02	0.25	6.14	0.52
tar	22.09	<0.001	21.56	<0.001	4.72	0.69	9.49	0.22
burnt rubber	16.79	0.02	22.95	<0.001	8.07	0.33	11.55	0.12
green	9.92	0.19	20.63	<0.001	11.21	0.13	15.88	0.03
vegetable	10.18	0.18	13.2	0.07	9.51	0.22	7.87	0.34
animal	24.12	<0.001	20.41	<0.001	8.23	0.31	2.38	0.94
woody	23.73	<0.001	10.04	0.19	27.58	<0.001	5.95	0.55
toasted	23.80	<0.001	7.44	0.38	24.39	<0.001	7.84	0.35
earthy	13.75	0.06	15.74	0.03	10.33	0.17	8.96	0.26
dusty	19.76	0.01	11.44	0.12	11.89	0.10	15.31	0.03

3.1 Shiraz

The data generated during the PM exercise was standardised for a correspondence analysis (CA) between the spiked samples of Shiraz (Figure 1) and the perception by the panel of odour attributes in partially de-aromatised wine. In the CA, the importance of a dimension (i.e., principal component) is reflected by its “eigenvalue” which indicates how much of the total inertia (i.e., variance) of the data is explained by this component (Abdi *et al*., 2013). In the Shiraz dataset, Dimension 1 (Dim 1) explains 49.85% of the inertia or variance. Dimension 2 explains a further 19.84 % of the inertia. For this cultivar, control samples C_ShA and C_Sh B are associated with the fruity/ sweet-associated attributes like ‘floral’, ‘berries’, ‘fruity, ‘spicy’ on the negative side of Dim 1. As Shiraz is known to have cultivar characteristics that are ‘berry’-associated,’ jammy’, ‘spicy’ (especially ‘black pepper’ spice), these results seem appropriate. As the volatile phenols were added to the samples the perceived fruity aspects of the samples changed. The samples spiked with single volatile phenols *o*-cresol (ShA_OC, and ShB_OC) are situated in the sensory space towards the middle of the plot, where the single spiked samples of 4-EP (ShA_4EP) and ShB_4EP) are also located. Attributes associated with the middle section of the CA include ‘ash’, ‘chemical’, and ‘plastic’. Samples that were spiked with both volatile phenols (ShA_OC+4-EP and ShB_OC+4-EP) are located on the positive side of Dim1, and aremost closely associated with the attributes ‘dusty/earthy’, ‘tar/BR’, ‘burnt rubber’ ‘vegetal’ and ‘green’. This is in line with the p-values given for the Cochran’s Q-test which show significant differences in these attributes, and also with work previously found in this study (Chapter 4 and 5) that indicate that binary combinations of volatile phenols can lead to negative attributes in Shiraz .

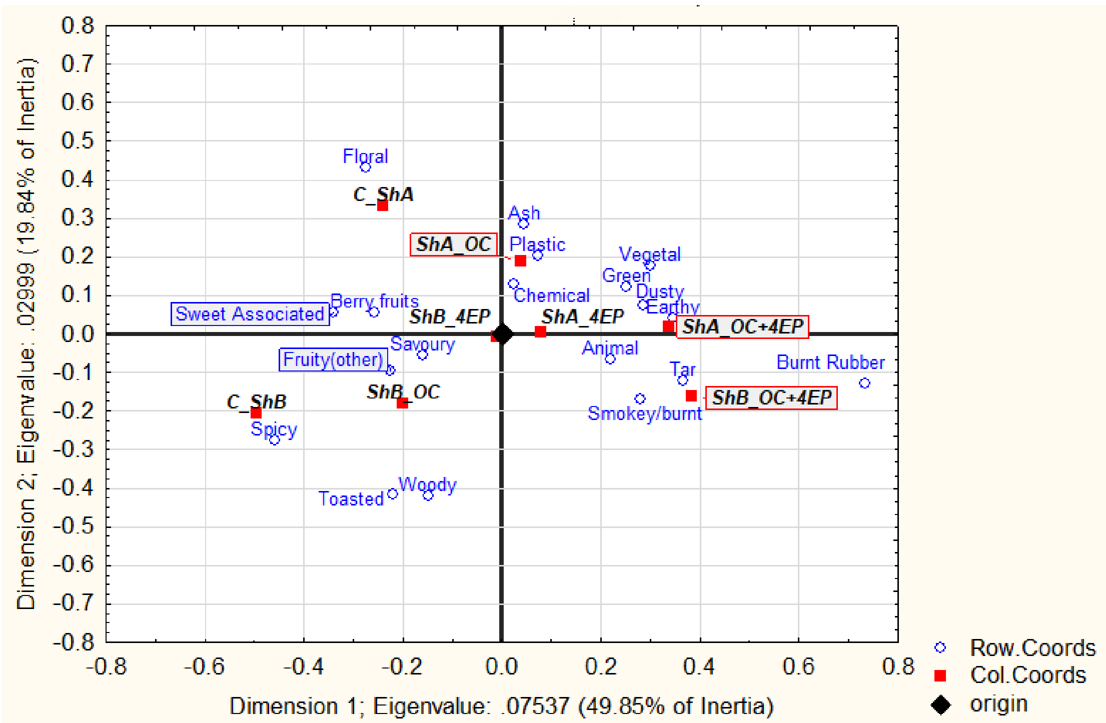


Figure 1. CA of Shiraz wine showing row and column coordinates (Dimension 1 x 2) for the eight Shiraz samples (control (C_ShA/B), o-cresol spiked (ShA/B_OC), 4-EP spiked (ShA/B_4EP) and binary spiked (ShA/B_OC+4EP)

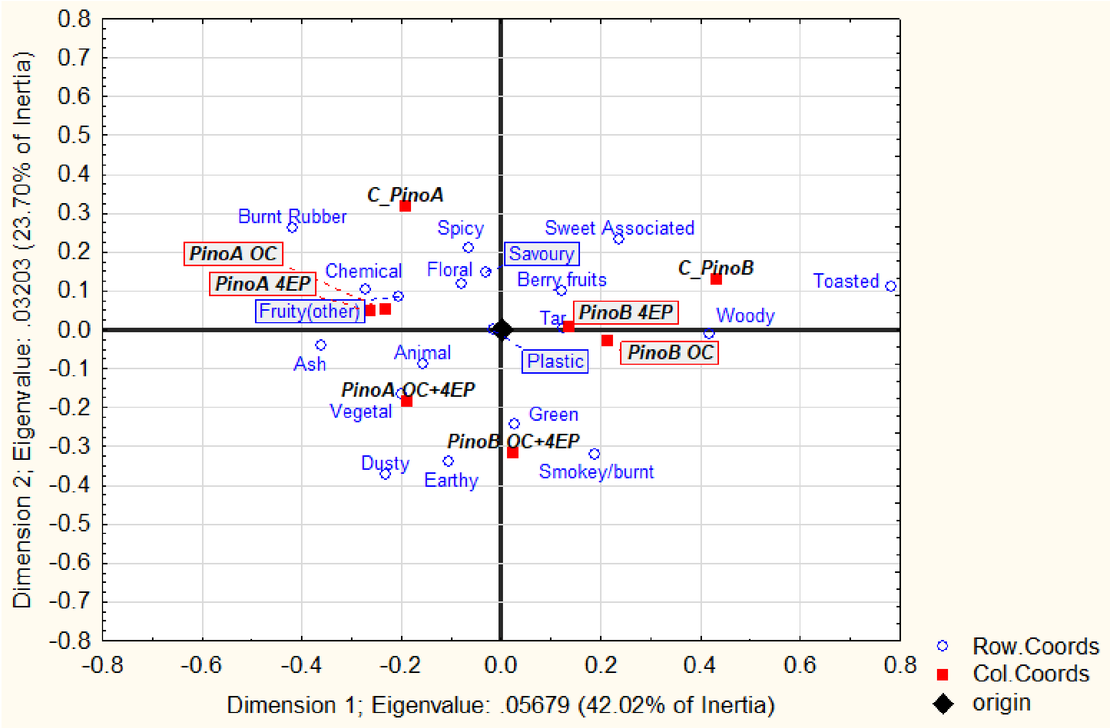


Figure 2. CA of Pinotage wine showing row and column coordinates (Dimension 1 x 2) and for eight Pinotage samples (control (C_PinoA/B), o-cresol spiked (PinoA/B_OC), 4-EP spiked (PinoA/B_4EP) and binary spiked (PinoA/B_OC+4EP)

3.2 Pinotage

Figure 2A shows the CA map between the spiked samples of Pinotage and the attributes perceived to be associated with control and spiked samples for this cultivar. Dim 1 in the CA explains 42% of the inertia in the data set. For Dim 2, a further 23 % of the inertia was explained, showing that the panel was well aligned regarding perception of attributes in the samples

Although the separation is not as clear for this cultivar as for Shiraz, there seems to be differences perceived in the sample set along Dimension 1 (Dim 1) according to the base wines (one of which was a bag-in-the-box wine blend from various areas (Pinotage A), and the other of which was a Wine of Origin Robertson wine (Pinotage B). There is much less separation between the PinoA samples concentrated in a small segment of Dim 1 (associated with 'vegetal', 'animal', 'chemical' and 'fruity-other') , than between PinoB samples which are widely scattered along the Dim 1 positive axis. This indicates that the volatile phenols had more effect on the odour profile of PinoB. The other control C_PinoA separates out towards 'burnt rubber', 'chemical' and 'leather'. The control sample C_PinoB is associated, on the positive side of Dim 1, with 'sweet-associated', 'berries', 'woody, and 'toasted' attributes. The spikes seem to enhance the natural attributes of the base wine, with the OC and 4-EP spikes in wine A associated with the perception of more negative attributes like 'chemical', 'leather', 'ashy', 'animal' and 'vegetal', and single spiked of volatile phenols in wine B associating with 'berry fruit', 'plastic', 'woody' attributes. Pinotage has been previously shown to have 'duco/nail varnish' qualities (Marais, 2003) and it is interesting that the presence of *o*-cresol seems to enhance this. Samples that were spiked with both volatile phenols (PinoA_OC+4EP and PinoB_OC+4EP) are closer to each other on the CA plot, separated along the second dimension from the control, and associated with 'dusty', 'earthy' and 'green' attributes. As Pinotage is not known to contain methoxypyrazine or green characteristics as a cultivar, this is also an interesting result and is in line with previous findings (Chapter 4 and 5) that indicate that binary combinations of volatile phenols can lead to unexpected results, including 'dusty', 'green' and 'tar/BR' attributes.

3.3 Merlot Noir

Figure 3A shows the correspondence analysis between the spiked samples of Merlot Noir and controls. Separation for the Merlot Noir wines is mainly along the Dim 1 between controls (C_MerlA, C_MerlB) closest to with sweet-associated and 'woody' attributes, and the samples spiked with both volatile phenols (MerA_OC+4EP, and MerB_OC+4EP). The latter samples are strongly associated with negative attributes: 'dusty', 'vegetal', 'smoky/burnt', 'green' and 'tar'. The binary spikes are also closest to the 'burnt rubber' attribute. Merlot Noir is know to naturally contain methoxypyrazines (Kotseridis *et al.*, 1998) as part of the primary aroma profile of the cultivar, and the presence of the volatile phenol seems to enhance the dusty and 'green' attributes perceived in these wines. Single spikes of 4-EP and *o*-cresol seem to cause the perception of attributes to shift from 'sweet-associated' and 'woody' descriptors towards the middle of the CA plot where attributes including 'ash', 'chemical', animal' and 'savoury' are found to dominate the aroma profile.

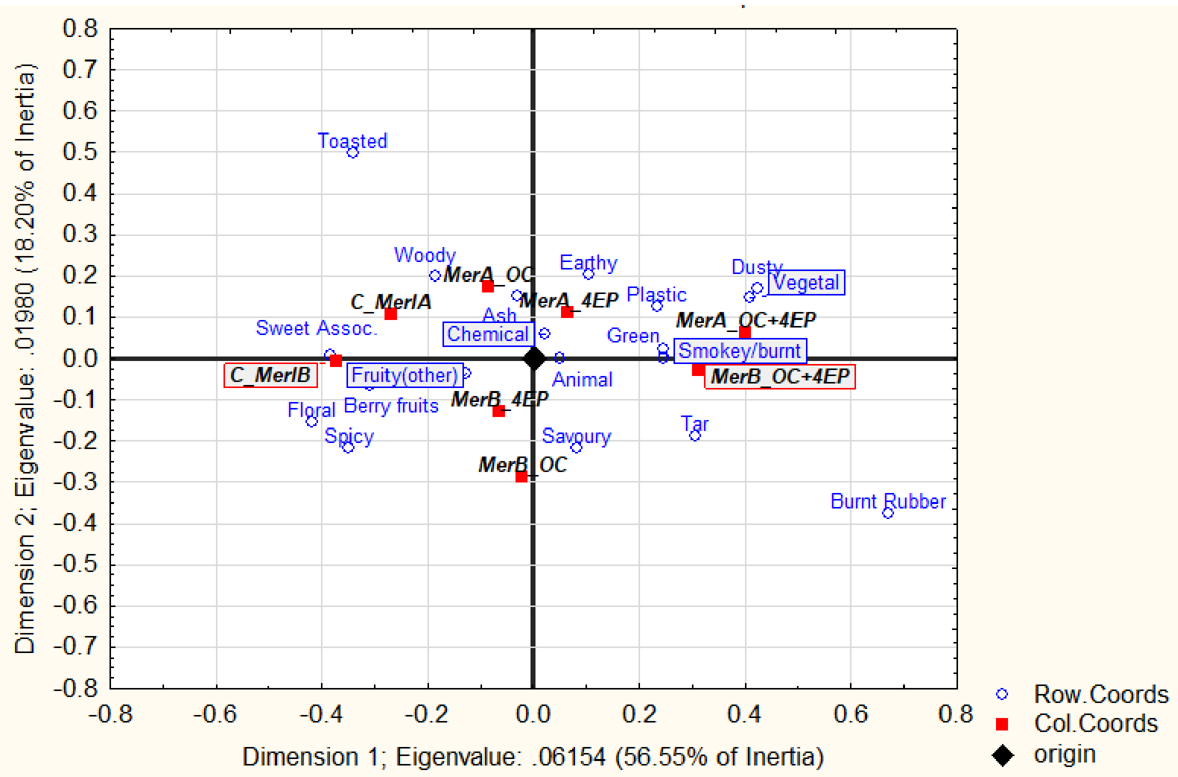


Figure 3. CA of Merlot Noir wine showing row and column coordinates (Dimension 1 x 2 for eight Merlot Noir samples (control (C_MerA/B), o-cresol spiked (MerA/B_OC), 4EP spiked (MerA/B_4EP) and binary spiked (MerA/B_OC+4EP) attributes associated with control and spiked samples for this cultivar. Dim 1 in the CA for Merlot shows an Eigen value of 0.061), explaining 56.55% of the inertia in the data set. For dimension 2, the variance was 0.02, explaining 18.2 % of the inertia.

3.4 Cabernet Sauvignon

Cabernet shows very similar trends in the perception of attributes associated with spikes to those of the cultivars described previously, with explained variance for Dim 1 accounting for 61.55% of the inertia, which is good separation and indicates the panel perceived clear differences between the samples. Dim 2 accounts for a further 18.15%. Control sample C_CSA is located in the quadrants with sweet/fruity descriptors such as ‘floral’, ‘berry fruits’, ‘fruity/other’, and ‘sweet-associated’. Control sample C_CSB is associated with ‘spicy’, ‘woody’ and ‘toasted’, as is CSB_OC, indicating that the spike of *o*-cresol has not had much effect on the perception of attributes in the spiked sample.

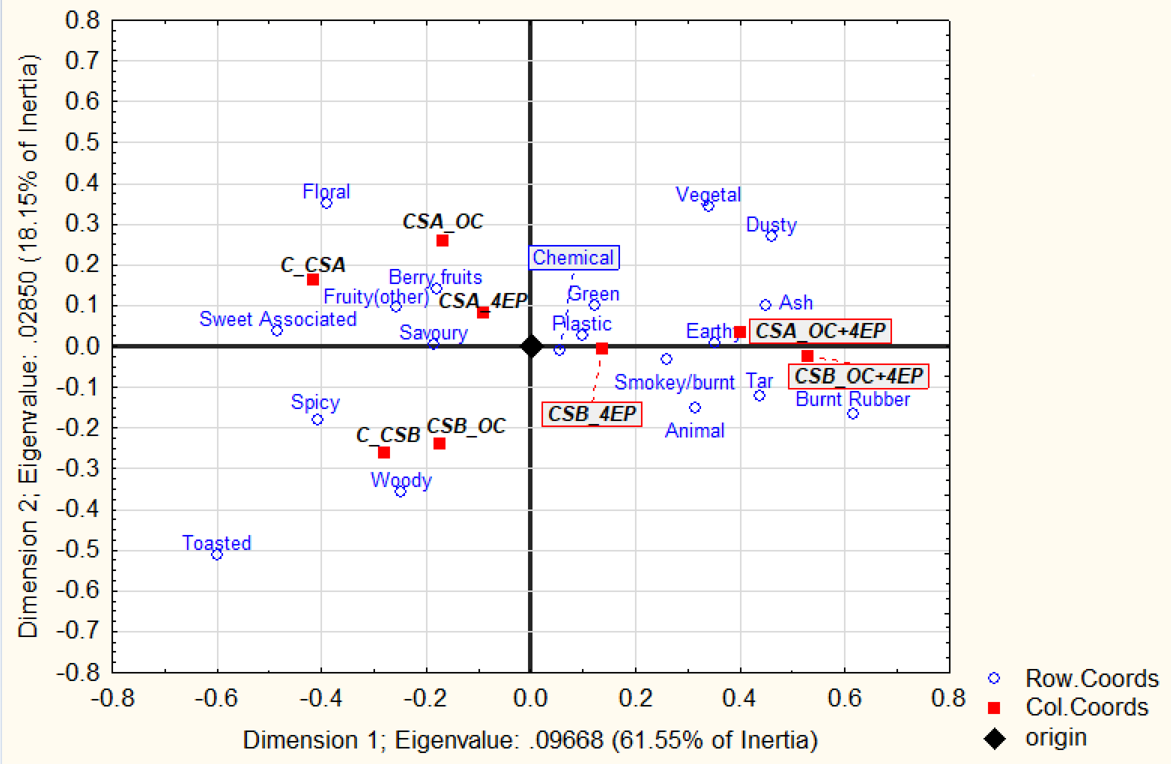


Figure 4. CA of Merlot Noir wine showing row and column coordinates (Dimension 1 x 2) for eight Cabernet Sauvignon samples (control (C_CSA/B), o-cresol spiked (CSA/B_OC), 4EP spiked (CSA/B_4EP) and binary spiked (CSA/B_OC+4EP)

Samples spiked with single compounds again occupy the sensory space in the centre of the CA, with CSA_OC and CSA_4EP associated with 'berry fruits' and 'fruity-other', but with CSB_4EP perceived as being closest to 'green', 'plastic' and 'leather'. The binary spikes in both base wines (CSA_OC+4EP and CSB_OC+4EP) are again associated with 'earthy', 'dusty', 'ashy', 'smoky-burnt', 'tar' and 'burnt rubber'.

4. Conclusions

Data presented here show that single and combinations of just two volatile phenols, *o*-cresol and 4-EP can have a marked effect on the perception of attributes in different cultivars. Two different wines from four commonly used cultivars in South African winemaking (viz. Cabernet Sauvignon, Merlot Noir, Pinotage and Shiraz) were spiked with single and binary combinations of two volatile phenols commonly associated with smoke-taint. Results of a sensory PM study showed that although there were marked similarities between all the cultivars for certain aspects, there were also subtle but important differences in sensory profiles for different cultivars. Clean controls were generally perceived as 'fruity berries', 'fruity-other', 'sweet-associated' and 'floral'. The controls in the case of Pinotage separated along Dim 1, and the base wine had a marked effect on the perception of spiked samples, with one sample showing fruity and woody characteristics and the other associated with 'spicy' and 'burnt rubber' attributes. There were differences between the cultivars in response to single volatile phenol spikes. In Cabernet Sauvignon and Merlot Noir, spikes of *o*-cresol could be perceived as fruity, or woody (generally pleasant and not dissimilar from the controls), but in Pinotage and Shiraz, the perception could be less positive and move towards 'savoury', 'plastic and chemical'. Generally, *o*-cresol did not seem to have a marked effect on perception on its own. Single spikes of 4-EP enhanced the 'green' character in Cabernet Sauvignon, but this was not a consistent effect. The binary spikes led to definite increases in the perceptions of 'tar', 'burnt rubber', 'dusty', 'earthy', 'smokey/burnt' and 'animal' across all four cultivars.

The most marked similarity of perceptual effect was in the binary spikes across all the cultivars. In Pinotage, the wines spiked with both VPs were associated with 'vegetal', 'smoky/burnt', 'green' and 'earthy' attributes, Merlot reacted similarly, with perception of 'vegetal', 'smoky' and 'tar' attributes enhanced. Shiraz also showed 'tar', 'burnt rubber', 'smoky' and 'animal' characteristics, and Cabernet Sauvignon exhibited 'ash', 'burnt rubber' and 'tar' attributes in binary spikes. It is clear from this study that volatile phenols on their own do not have as marked effect as in they do in combination. As they are unlikely to occur as separate compounds in the winemaking milieu, this study crucially shows the effect of low levels of volatile phenols on causing or exaggerating negative odour attributes in red wines of different cultivars, and the need to manage winemaking carefully if there is a risk that grapes or wine have been exposed. The work also emphasises the need for researchers to vigilantly consider the composition of the delivery matrix when determining and comparing odour threshold estimates for volatile phenols.

REFERENCES

Chaput, M.A., El Mountassir, F., *et al*., 2012. Interactions of odorants with olfactory receptors and receptor neurons match the perceptual dynamics observed for woody and fruity odorant mixtures Eur. J. Neurosci. 35, 4, 584–597.

Goldner, M.C. Zamore, M., Di Leo Lira, P., *et al*., 2009. Effects of ethanol level in the perception of aromaattributes and the volatile composition of red wines J. Sens. Stud. 24, 2, 243–257.

Goode, J.,2018. Lower/ reduced alcohol wines. http://www.wineanorak.com/loweralcoholwines.htm.

Grosch, W., 2001. Evaluation of the Key Odorants of Foods by Dilution Experiments, Aroma Models and Omission Chem. Senses 26, 5, 533–545.

Kotseridis, Y., Anocibar Beloqui, A., *et al*., 1998. An analytical method for studying the volatile compounds of Merlot Noir clone wines Am. J. Enol. Vitic. 49, 1, 44–48.

Laing, D.G., Eddy, A., *et al*., 1994. Perceptual characteristics of binary, trinary, and quaternary odor mixtures consisting of unpleasant constituents Physiol. Behav. 56, 1, 81–93.

Macfie, H.J., Brathchell, N., *et al*., 1989. Designs to balance the effect of order of presentation and first-order carry-over effects in Hall Tests J. Sens. Stud. 4, 2, 129–148.

Mafata, M., Buica, A., *et al*., 2018. The Effect of Grape Temperature on the Sensory Perception of Méthode Cap Classique Wines South African J. Enol. Vitic. 39, 1, 132–140.

Marais, J., 2003. Effect of Different Wine-Making Techniques on the Composition and Quality of Pinotage Wine. I. Low-Temperature Skin Contact Prior to Fermentation South African J. Enol. Vitic. 24, 2, 70–75.

De March, C.A., Ryu, S.E., *et al*., 2015. Structure-odour relationships reviewed in the postgenomic era Flavour Frag. J. 30, 5, 342–361.

Ozturk, B. & Anli, E., 2014 Different techniques for reducing alcohol levels in wine: A review. BioWeb of Conferences, 3. https://www.bio-conferences.org/articles/bioconf/pdf/2014/02/bioconf_oiv2014_02012.pdf

Perry, D.M. & Hayes, J.E., 2016. Effects of matrix composition on detection threshold estimates for Methyl Anthranilate and 2-Aminoacetophenone Foods 5, 2, 35–45.

Petrozziello, M., Asproudi, A., *et al*., 2014. Influence of the matrix composition on the volatility and sensory perception of 4-ethylphenol and 4-ethylguaiacol in model wine solutions. Food Chem. 149, 197–202.

Rospars, J.-P., Lansky, P., *et al*., 2008. Competitive and Noncompetitive Odorant Interactions in the Early Neural Coding of Odorant Mixtures J. Neurosci. 28, 10, 2659–2666.

de Vries, C.J., Mokwena, L.M., *et al*., 2016. Determination of Volatile Phenol in Cabernet Sauvignon Wines, Made from Smoke-affected Grapes, by using HS-SPME GC-MS. South African J. Enol. Vitic. 37, 1, 15–21.

APPENDIX A:

Table 1: Spiking regime for matrix experiments for four cultivars (two wines of each cultivar) post partial de- aromatization

Shiraz A 2017				
	o-cresol [Required] µg/l	Spike *o*-cresol/ 500ml	4EP [required] µg/l	Spike 4EP/ 500ml
[Spiking stock solution]		100ppm		1000ppm
Original amounts in sample		5µg/l		3µg/l
ShA_Control	0	-	0	-
ShA_OC	62	285µl	0	-
ShA_4EP	0	-	605	301µl
ShA_OC+4EP	62	285µl	605	301µl
Shiraz B: 2017				
Original amounts in sample		14µg/l		1µg/l
ShB_Control	0	-	0	-
ShB_OC	62	240µl	0	-
ShB_4EP	0	-	605	302µl
ShB_OC+4EP	62	240µl	605	302µl
Pinotage A 2016				
Original amounts in sample		7µg/l		3µg/l
PinoA_Control	0	-	0	-
PinoA_OC	62	275µl	0	-
PinoA_4EP	0	-	605	301µl
PinoA_OC+4EP	62	275µl	605	301µl
Pinotage B: 2017				
Original amounts in sample		13µg/l		1µg/l
PinoB_Control	0	-	0	-
PinoB_OC	62	250µl	0	-
PinoB_4EP	0	-	605	302µl
PinoB_OC+4EP	62	250µl	605	302µl
Merlot noir A 2017				
Original amounts in sample		5µg/l		0µg/l
MerA_Control	0	-	0	-
MerA_OC	62	285µl	0	-
MerA_4EP	0	-	605	303µl
MerA_OC+4EP	62	285µl	605	303µl
Merlot noir B: 2017				
Original amounts in sample		5µg/l		55µg/l
MerB_Control	0	-	0	-
MerB_OC	62	285µl	0	-
MerB_4EP	0	-	605	275µl
MerB_OC+4EP	62	285µl	605	275µl
Cabernet Sauvignon A 2017				
Original amounts in sample		10µg/l		3µg/l
CSA_Control	0	-	0	-
CSA_OC	62	260µl	0	-
CSA_4EP	0	-	605	301µl
CSA_OC+4EP	62	260µl	605	301µl
Cabernet Sauvignon B 2016				
Original amounts in sample		6µg/l		1µg/l
CSB_Control	0	-	0	-
CSB_OC	62	280µl	0	-
CSB_4EP	0	-	605	302µl
CSB_OC+4EP	62	280µl	605	302µl

Table 2: Attribute list for PM

Descriptor to use	For Attribute
Berry fruits	blackberry
	blackcurrant
	red berries
	strawberry
	cherry
	jammy
Fruity (other)	mango
	banana
	prunes
	raisins
	artificial fruit
	peach
	plums
	melon
	stewed fruit
	bruised apple
	tobacco
	quince jelly
Sweet Associated	marmalade
	toffee/ fudge
	vanilla
	caramel
	chocolate
	cocoa
	port
	molasses/ burnt sugar
Floral	perfume
	elderflower
	floral
	muscat
	rose
	soapy
	violets
Spicy	liquorice
	spicy/ spice
	anise/ aniseed
	coriander
Savoury	bacon
	meaty
	soy sauce
	balsalmic
	savoury
Smokey/burnt	smoke
	ashtray

	burnt
	ashy
Chemical/plastic	moth balls
	Doom
	solvent
	chemical
	floor polish
	medicinal
	alcohol
	rubbery
	shoe polish
	plastic
Tar/burnt rubber	tar
	acrid
	Jeyes fluid
	burnt rubber
	creosote
Green/vegetal	greenpepper
	vegetal
	olives
	potato skin
	green wood
	green
	herbaceous
Animal	wet dog
	centipede/ shongololo
	blood
	leather
	musk
	barnyard
Woody/Toasted	coffee
	oak
	pencil shavings
	mocha
	coconut
	nutty
	planky/sawdust
Earthy/dusty	dusty
	earthy
	mouldy
	wet stone/gravel
	chalky
	musty

Table 2 Cochran's Q-test for Shiraz results

	Subject variable=judge/rep				
	independent var	response (dichotomous) var	Q-statistics	df	p-val
1	wine sample	Berry fruits	21,52	7	0
2	wine sample	Fruity(other)	18,2	7	0,01
3	wine sample	Sweet Associated	18,39	7	0,01
4	wine sample	Floral	20,32	7	0
5	wine sample	Spicy	17,1	7	0,02
6	wine sample	Savoury	5,98	7	0,54
7	wine sample	Smokey/burnt	23	7	0
8	wine sample	Ash	10,23	7	0,18
9	wine sample	Chemical	10,46	7	0,16
10	wine sample	Plastic	7,64	7	0,37
11	wine sample	Tar	21,56	7	0
12	wine sample	Burnt Rubber	22,95	7	0
13	wine sample	Green	20,63	7	0
14	wine sample	Vegetal	13,2	7	0,07
15	wine sample	Animal	20,41	7	0
16	wine sample	Woody	10,04	7	0,19
17	wine sample	Toasted	7,44	7	0,38
18	wine sample	Earthy	15,75	7	0,03
19	wine sample	Dusty	11,44	7	0,12

Table 3: Cochran's Q-test for Pinotage results

	Subject variable=judge/rep				
	independent var	response (dichotomous) var	Q-statistics	df	p-val
1	Wine ID	Berry fruits	8,69	7	0,28
2	Wine ID	Fruity(other)	12,41	7	0,09
3	Wine ID	Sweet Associated	21,06	7	0
4	Wine ID	Floral	5,27	7	0,63
5	Wine ID	Spicy	5,28	7	0,63
6	Wine ID	Savoury	16,77	7	0,02
7	Wine ID	Smokey/burnt	25,04	7	0
8	Wine ID	Ash	8,71	7	0,27
9	Wine ID	Chemical	18,45	7	0,01
10	Wine ID	Plastic	9,02	7	0,25
11	Wine ID	Tar	4,72	7	0,69
12	Wine ID	Burnt Rubber	8,07	7	0,33
13	Wine ID	Green	11,21	7	0,13
14	Wine ID	Vegetal	9,51	7	0,22
15	Wine ID	Animal	8,23	7	0,31
16	Wine ID	Woody	27,58	7	0
17	Wine ID	Toasted	24,39	7	0
18	Wine ID	Earthy	10,33	7	0,17
19	Wine ID	Dusty	11,89	7	0,1

Table 4: Cochran's Q-test for Merlot Noir

	Subject variable=judge/rep				
	independent var	response (dichotomous) var	Q-statistics	df	p-val
1	Wine sample	Berry fruits	17,72	7	0,01
2	Wine sample	Fruity(other)	5,47	7	0,6
3	Wine sample	Sweet Assoc.	22,38	7	0
4	Wine sample	Floral	13,66	7	0,06
5	Wine sample	Spicy	7,18	7	0,41
6	Wine sample	Savoury	8,42	7	0,3
7	Wine sample	Smokey/burnt	17,93	7	0,01
8	Wine sample	Ash	9,85	7	0,2
9	Wine sample	Chemical	4,58	7	0,71
10	Wine sample	Plastic	6,14	7	0,52
11	Wine sample	Tar	9,49	7	0,22
12	Wine sample	Burnt Rubber	11,55	7	0,12
13	Wine sample	Green	15,88	7	0,03
14	Wine sample	Vegetal	7,87	7	0,34
15	Wine sample	Animal	2,38	7	0,94
16	Wine sample	Woody	5,95	7	0,55
17	Wine sample	Toasted	7,84	7	0,35
18	Wine sample	Earthy	8,96	7	0,26
19	Wine sample	Dusty	15,31	7	0,03

Table 5: Cochran's Q-test for Cabernet Sauvignon

	Subject variable=judge/rep				
	independent var	response (dichotomous) var	Q-statistics	df	p-val
1	Wine sample	Berry fruits	6,89	7	0,44
2	Wine sample	Fruity(other)	19,05	7	0,01
3	Wine sample	Sweet Associated	38,89	7	0
4	Wine sample	Floral	16,02	7	0,02
5	Wine sample	Spicy	6,05	7	0,53
6	Wine sample	Savoury	13	7	0,07
7	Wine sample	Smokey/burnt	17,38	7	0,02
8	Wine sample	Ash	8,73	7	0,27
9	Wine sample	Chemical	11,41	7	0,12
10	Wine sample	Plastic	2,92	7	0,89
11	Wine sample	Tar	22,09	7	0
12	Wine sample	Burnt Rubber	16,79	7	0,02
13	Wine sample	Green	9,92	7	0,19
14	Wine sample	Vegetal	10,18	7	0,18
15	Wine sample	Animal	24,12	7	0
16	Wine sample	Woody	23,73	7	0
17	Wine sample	Toasted	23,8	7	0
18	Wine sample	Earthy	13,75	7	0,06
19	Wine sample	Dusty	19,76	7	0,01

Chapter 9

General discussion and conclusions

General discussion and conclusions

With climate change, heatwaves, and more frequent forest fires across the world (Lucas *et al.*, 2007; Jiranek, 2011; Strydom & Savage, 2016; Wolf, 2018), the effects of smoke on agricultural crops are viewed as increasingly important. When wine is made from smoke-affected grapes, smoke volatiles are transferred from the grapes during the winemaking process, and manifest in the wine as so-called "smoke taint". Providing wine producers with insight into the sources, effects and amelioration of smoke taint is therefore crucial in supporting the wine industry in improving the quality of its products. Volatile phenols (VPs) are a group of compounds that are derived from a number of sources including oakwood, microbial fermentations (specifically *Brettanomycesl),* but have become associated with off-flavours in wine made from smoke-affected grapes. VPs are generally accepted as being benign to wine aroma at sub-threshold levels (Boidron *et al*., 1988; Prida & Chatonnet, 2010), but are known to contribute to a continuum of smoke taint related off-flavours including 'burnt', 'bretty', 'smoky', and 'ashy' attributes in wine at peri- or infra-threshold levels (Jiranek, 2011; Kennison, Ward, *et al.*, 2011). A number of studies (Kennison *et al.*, 2009; Kennison, Wilkinson, *et al.*, 2011; Ristic *et al.*, 2015, 2016) have addressed the effect of smoke taint on deliberately/experimentally 'smoked' grapes and wine. Aroma compounds will be quantified by chemical/analytical means and compare levels to odour detection thresholds (ODTs) provided by scientific literature (Ristic *et al.*, 2011; Kelly *et al.*, 2012, 2014; Kennison, 2013). In other studies, it has been found that sensory results do not always correlate well with predictions that are based on the chemistry of the solution (Villamor & Ross, 2013; Lapalus, 2016; Wilson *et al*., 2018).

The first aim of the thesis was to investigate the issue of the effects of VPs in commercial samples of twelve potentially smoke-affected South African red wines from various Wine of Origin regions around the Western Cape. Results are presented in Chapter 3. Chemical analysis of a range of twelve VPs by GC-MS was conducted, and the same wines were characterised with Descriptive Analysis (DA) using a trained panel. Using the combined dataset, it was clear that that concentration and composition of VPs correlated with certain sensory attributes in the wines. The links between VPs and specific off-odours were demonstrated. Wines with very low levels of VPs showed fruity and sweet-associated characteristics, and those with supra-threshold levels showed negative attributes. Historical data for bushfire events around winegrowing areas of South Africa showed associations between negative attributes and historical bushfire events near the vineyards in the period leading up to harvest, which has not been previously demonstrated. In some cases, sensory effects ('earthy/dusty/potato skin', 'mouldy/musty' and 'cooked veg') could not be attributed to peri- or supra-threshold concentrations of VPs, but may have been due to combinations of volatile phenols at subthreshold levels, possibly influenced by the presence of other compounds. Given that the samples were submitted as potentially smoke tainted, and volatile phenol levels were high in only three of the wines, it seems there is scope for South African winemakers (and likely winemakers globally) to be better trained in identifying smoke taint via sensory evaluation. A significant limitation of this study was the fact that there was little information available on the wines as far as winemaking treatments or phenological stage of any fire events during the ripening of the grapes. These shortcomings will be need to addressed in future studies of this nature.

It was decided,as a result of the first study, to scrutinise perceptual olfactory effects at low levels, of five compounds associated with off-flavours in red wine. These were of three VPs: guaiacol, *ortho*-cresol (*o*-cresol), 4-ethylphenol (4-EP), 3- isobutyl-2-methoxypyrazine (IBMP) and 2,4,6-trichloroanisole (TCA).

Ideally, the threshold for each individual ODT should be determined in the study matrix for every study. Unfortunately, formal sensory studies are known to be complex, time-consuming and expensive (Valentin *et al.*, 2012) and are unsurprisingly scarce in the wine literature, particularly in establishing ODTs (Czerny *et al.*, 2008). It was necessary to first establish if ODTs provided in the literature were appropriate in the study matrix, which was de-aromatised red wine as recommended by (Pineau & Barbe, 2009). ODTs are often determined in matrices (for example, water) that are dissimilar from the new study matrix (for example, white wine), which lead to inappropriate actions and conclusions on the part of researchers. The second aim of the thesis was thus to test a pragmatic sensory approach would be a useful addition to current wine research. Chapter 4 describes such an approach to verifying the ODTs for the five aroma compounds listed above in a partially de-aromatised Shiraz red wine, using a simplified version ASTM E-679-04 triangle test. Simultaneously, the sensitivity of potential sensory panel members to compounds at ODT level was investigated to establish if previous training in sensory evaluation of wine influenced panellists' abilities to distinguish threshold differences between samples and controls. Results showed that judges were better able to detect compounds if they had prior experience or training in wine evaluation training, and that overall they were able to distinguish samples spiked with VPs as significantly different to controls at the $p < 0.01$ level. As IBMP and TCA were the last two compounds to be assessed of the five, and also the two compounds that showed the least correct responses or differences between experienced and inexperienced judges, it is probable that the assessors experienced sensory fatigue. This is a limitation of this strategy, and if the study were to be repeated, it would be advisable for each compound to be tested on a different day at different levels, or presented in a random order to the judges. Despite the limitations, this simplified approach met the aims of the research, and provides information that should be useful when carrying out sensory studies with fairly limited resources and within tight timelines. It also provides helpful information on panel members and detection thresholds for a red wine matrix.

Although anecdotal data suggests that these compounds contribute to 'acrid' and 'green-associated'attributes, there are no studies, to our knowledge, focused on olfactory perception of interactions between VPs and other taint-causing compounds like IBMP and TCA. Knowledge of the chemical and sensory effects in wines containing VPs in combination with these compounds would give much greater understanding of the nature of off-flavours. Since these compounds often occur in a wine simultaneously, and very little work has been carried out the third aim of the thesis was to characterise the perceptual olfactory interactions caused by combinations of the five odour-active compounds mentioned above. Using a D-optimal fractional statistical design and DA, perceptual olfactory interactions of two, three, four and five off-flavour compounds spiked into red wine were characterised to elucidate new sensory attributes resulting from perceptual blending in complex mixtures of components. This type of perceptual interaction research in wine has not been done on this scale before. In Chapter 5, the results of the first part of this study in a partially de-aromatised red wine matrix are shown. The data showed that the olfactory outcome of binary mixtures in red wine cannot be predicted from the attributes of single compounds.. Results indicated that certain attributes ('cooked vegetables', 'black pepper', 'mouldy/musty', 'pencil shavings', 'smoky', 'soy sauce', 'plums') were perceived by panellists only in the samples with individual compounds. The subthreshold level of a compound was sufficient to induce significant interactions and effects in some cases, and these were also linked to positive attributes (for example, guaiacol and o-cresol . Binary systems generated different descriptors ('ashtray', 'plastic/chemical', 'tar/BR' and 'savoury'). but the key influence on perception of wine aroma was the presence or absence of IBMP. The 'ashy/ ashtray/ ashiness' attribute, known to be associated with smoke taint, was affected significantly by IBMP at peri-threshold levels. It was also clear that VPs contributed in some cases to the 'green' aromas on the odour continuum. Chapter 6, the second interaction study, also addressed this aim, investigating the perceptual effects of larger combinations. The results showed the clear olfactory opposition between clean controls, wines spiked with single compounds (generally fruity and sweet- associated), and wines spiked with complex combinations (3 to 5 off-flavour compounds). Samples with a greater number of spiked VPs were linked to negative attributes 'earthy/ dusty/potato skin', 'mouldy/musty', 'herbaceous', 'ashtray', 'tar/BR', 'rubber/chemical' and 'medicinal/Elastoplast™', thus showing that new sensory attributes can result from perceptual blending in complex mixtures of components . These interesting and novel results encourage further volatile phenol/ taint compound studies to explore additional interactions.

Chapter 7 demonstrates another pragmatic approach, using Projective Mapping (PM) with a large sample size (n=18) than has been previously tested in the literature. The study aim was to explore the use of rapid sensory methods in interaction studies for volatile phenols and validate the use of PM for characterising combinations of off-odour compounds in a red wine matrix, by comparing results to those of a similar study using DA. Indeed, very similar results to the DA interaction study for four compounds in red wine were shown. Samples containing only volatile phenols (VPs) separated from samples containing combinations of VPs and IBMP in both the DA and the PM sensory tests. Correspondence analysis showed clearly that a comparable set of descriptors was generated. These results thus confirm that related work on VPs in red wine matrices can be carried out with a trained panel using PM rather than DA in future studies.

The review of the literature showed that a significant gap in peer-reviewed scientific knowledge as the characterization of compounds or assignation of threshold values as a result of a formal determination study in different wine matrices. The final aim of this work was therefore to establish whether there were perceptual olfactory effects that were common to all cultivars, or whether the matrices responded differently from an aroma perception perspective. Chapter 8 reports effects of combinations of two VPs on four cultivars. The qualitative effects of two wine off-flavour contributors, 4-EP and *o*-cresol, were tested in combination in four different cultivars (i.e. Cabernet Sauvignon, Merlot Noir, Pinotage and Shiraz) using PM. The study showed that *o*-cresol and 4-EP can have a marked effect on the perception of attributes in different cultivars. Although there were similarities between the cultivars for certain aspects, there were also subtle but important differences in sensory profiles of the spiked samples, for example, increases in the perceptions of 'animal', 'tar', 'burnt rubber', 'dusty' 'earthy' across all four cultivars in samples with binary spikes. Differences in the response of base wine to spikes, particularly with Pinotage, were shown. It is clear from this study that, independent of cultivar, individual VPs do not have as marked effect as in they do in combination. As VPs are unlikely to occur as separate compounds in the winemaking environment, this study crucially shows the effect of low levels of VPs on causing or exaggerating negative odour attributes in red wines.

Although the sensory panel in this study proved admirably reliable and sensitive, it is certainly the case that this, and other sensory studies, are limited by the cost and complex organization around panel participation. Rapid methods may have provided faster answers earlier on, and led to better decision making around spiking levels and choice of compounds. Also, as descriptive analysis is driven by panel consensus, attributes sometimes varied, and a more consistent lexicon would have reduced variability, and may have revealed more intense differences. In the smoke taint assessment of twelve wines, a prescreening of the samples would have meant that wines that were actually smoke tainted were selected. The de-aromatised matrix, though a good base for the work, and providing a reasonable 'canvas' on which to determine interactions, is certainly not representative of all the myriad red wine matrices in industry. Although there is not a lot of information in the literature, a very careful selection of compounds based on specific additive or subtractive olfactory criteria could have given more useful data.

Perceptual interaction phenomena between aroma compounds in red wines represent an important source of complexity, and emphasise the importance of the matrix and other compounds in solution when carrying out sensory studies. Crucially, low levels of VPs may cause or exaggerate negative odour attributes in red wines, which has implications for winemaking with smoke-affected grapes. This work, which generates new knowledge is areas that have been little explored in the wine literature, may ultimately help inform winemaking decisions, particularly when dealing with cultivars that naturally have higher levels of methoxypyrazines, like Merlot Noir and Cabernet Sauvignon.

Additional work should include a study investigating the effects of VPs and other taint compounds on style and/or cultivar typicality, as there are few studies currently addressing this topic in the literature. Exploring rapid/pragmatic ways of training and demonstrating these interactions to the people in the industry for rapid “diagnosis” of wines affected by smoke taint or other off-flavours would lead to better decision-making in the cellar. Winemakers would be better able to rapidly assess the necessity for, and the efficacy of, amelioration treatments. Training and experience would certainly assist in preventing the misidentification of smoke taint, and the associated economic implications, as the effects threshold and sub- threshold levels of volatile phenols may not significant detrimental effects on wine aroma profiles (and therefore wine quality), where fruit attributes are prominent. This training is important because, as has been confirmed during this study, repeated exposure leads to experience which leads to reproducible and reliable success in detection of off-flavours. The unique and complex interactions between language, training, and olfaction should definitely be assessed in future studies.

REFERENCES

Boidron, J., Chatonnet, P., *et al*., 1988. Influence du bois sur certaines substances odorantes des vins Connaiss. la vigne du vin 22, 4, 275–294.

Czerny, M., Christlbauer, M., *et al*., 2008. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions Eur. Food Res. Technol. 228, 2, 265–273.

Jiranek, V., 2011. Editorial: Smoke taint compounds in wine: Nature, origin, measurement and amelioration of affected wines Aust. J. Grape Wine Res. 17, S2–S4.

Kelly, D., Zerihun, A., *et al*., 2012. Exposure of grapes to smoke of vegetation with varying lignin composition and accretion of lignin derived putative smoke taint compounds in wine Food Chem. 135, 2, 787–798.

Kelly, D., Zerihun, A., *et al*., 2014. Winemaking practice affects the extraction of smoke-borne phenols from grapes into wines Aust. J. Grape Wine Res. 20, 3.

Kennison, K., Wilkinson, K., *et al*., 2009. Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine Aust. J. Grape Wine Res. 15, 228–237.

Kennison, K., 2013. Effect of smoke in grape and wine production Gov. West. Aust. Dep. Agric. Food Bull. Bulletin 4 . https://www.awri.com.au/wp-content/uploads/smoke_effect_in_grapes_and_wine.pdf

Kennison, K., Wilkinson, K., *et al*., 2011. Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties Aust. J. Grape Wine Res. 17, 2, S5–S12.

Lapalus, E., 2016. Linking sensory attributes to selected aroma compounds in South African Cabernet Sauvignon wines. Master’s thesis, Stellenbosch University, Western Province, South Africa

Lucas, C., Hennessy, K., *et al*., 2007. Bushfire Weather in Southeast Australia: Recent Trends and Projected Climate Change Impacts, Consultancy Report prepared for The Climate Institute of Australia Bushfire CRC and Australian Bureau of Meteorology; * CSIRO Marine and Atmospheric Research, Melbourne.

Pineau, B. & Barbe, J., 2009. Examples of Perceptive Interactions Involved in Specific " Red- " and " Black-berry " Aromas in Red Wines J. Agric. Food Chem. 57, 9, 3702–3708.

Prida, A. & Chatonnet, P., 2010. Impact of oak-derived compounds on olfactory perception of barrel-aged wines Am. J. Enol. Vitic. 50, 4, 447–455.

Ristic, R., Osidacz, P., *et al*., 2011. The effect of winemaking techniques on the intensity of smoke taint in wine Aust. J. Grape Wine Res. 17, 2, 29–40.

Ristic, R., Boss, P., *et al*., 2015. Influence of Fruit Maturity at Harvest on the Intensity of Smoke Taint in Wine Molecules 20, 5, 8913–8927.

Ristic, R., Fudge, A., *et al*., 2016. Impact of grapevine exposure to smoke on vine physiology and the composition and sensory properties of wine Theor. Exp. Plant Physiol. 28, 1, 67–83.

Strydom, S. & Savage, M., 2016. A spatio-temporal analysis of fires in South Africa South African J. Sci. J Sci 112, 11, 1–8.

Valentin, D., Chollet, S., *et al*., 2012. Quick and dirty but still pretty good: a review of new descriptive methods in food science Int. J. Food Sci. Technol. 47, 8, 1563–1578.

Villamor, R. & Ross, C., 2013. Wine Matrix Compounds Affect Perception of Wine Aromas Annu. Rev. Food Sci. Technol. 4, 1, 1–20.

Wilson, C., Brand, J., *et al*., 2018. Interaction Effects of 3-Mercaptohexan-1-ol (3MH), Linalool and Ethyl Hexanoate on the Aromatic Profile of South African Dry Chenin blanc Wine by Descriptive Analysis (DA) South African J. Enol. Vitic. 39, 2, 271–283.

Wolf, L., 2018. Wildfires and wine: A detective story. Chem. Eng. News 96, 19, 22–25.